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(54) Title: TUMOR TARGETING WITH L-ENANTIOMERIC OLIGONUCLEOTIDE CONJUGATES OF IMMUNOREAGENTS AND OF CHELATED RADIONUCLIDES

(57) Abstract

The present invention is directed to a non-radioactive targeting immunoreagent that comprises an immunoreactive group, one or more non-self-associating L-enantiomeric oligonucleotide sequences, and one or more linking groups, and to a radioactive targeting immunoreagent that comprises an L-enantiomeric oligonucleotide sequence that is complementary in sequence to and capable of hybridization with one or more fragments of a non-self-associating L-enantiomeric oligonucleotide sequence, one or more chelating agents, one or more linking groups, and one or more radionuclides. The present invention is also directed to pharmaceutical compositions comprising one or more of the above-described immunoreagents and a pharmaceutically acceptable carrier. The present invention is further directed to methods for treating and imaging disease sites in patients.

TUMOR TARGETING WITH L-ENANTIOMERIC
OLIGONUCLEOTIDE CONJUGATES OF
IMMUNOREAGENTS AND OF CHELATED RADIONUCLIDES

Field of the Invention

This invention relates to sequential targeting and delivery of immunoreagent compositions which find particular utility in the therapy and diagnostic imaging of cancer by means of a tumor targeted sequential delivery system comprising a primary non-radioactive targeting immunoreagent and a secondary radioactive delivery agent. This invention also relates to novel methods for the attachment of L-enantiomeric oligonucleotides, complementary L-enantiomeric oligonucleotides, and chelates, to proteins, and to bifurcated tumor targeting and delivery vectors for the treatment and diagnostic imaging of tumors.

Background of the Invention

The various, currently available, radiolabeled immunoreactive proteins and methods which are employed in diagnostic imaging and targeted therapeutic applications suffer from certain disadvantages. For example, radioimmunotherapy and diagnostic imaging with the various currently available radiopharmaceuticals which include radionuclide-containing immunoreactive proteins can be less than optimal because these radiopharmaceuticals may bind to non-target normal tissue. This binding can result in undesirable toxicity to normal tissue during therapeutic applications as well as in high background signals during diagnostic imaging applications. Inefficient covalent bonding of the radioactive component with protein in conjugate preparation can be another problem due to release of the radioactive component which may then deposit in healthy tissue. Also, long plasma half-lives of currently available radionuclide-containing immunoreactiv

proteins and slow clearance of radionuclide from the
body can result in prolonged exposure of normal tissue
to damaging effects of radiation and can produce
unacceptable toxic effects in otherwise normal and
5   disease free tissues in the body, especially in those
tissues and cells most sensitive to radiation damage,
e.g., the stem cells of the bone marrow and
gastrointestinal tract. While the number of ionic
radionuclides that can be associated with an
10   immunoreactive protein is restricted by the number of
sites of chelation available, an increase in that number
which can be achieved by increasing the number of
chelating agents conjugated to the protein can produce a
decrease in the immunoreactivity of the protein. This
15   can limit the number of such chelating agents that can
be attached to the protein. The number of chelating
agents that can be attached to an immunoreactive protein
is also limited by the number of available groups such
as, for example, amino groups suitable for use in
20   attachment of the chelating agents and by the potential
immunogenicity of the thus modified protein which, being
highly derivatized, could be recognized by a host immune
system as being haptenated.
        It is an object of the present invention to
25   overcome some of the aforementioned disadvantages of the
currently available radiolabeled immunoreactive
proteins.
        Nucleic acids in the form of deoxyribonucleic acid
(DNA) and ribonucleic acid (RNA) encode and transfer
30   genetic information for the cellular synthesis of
proteins and enzymes. Naturally occuring nucleic acids
are composed of nucleosides such as 2'-deoxyadenosine
(dA), 2'-deoxyguanosine (dG), 2'-deoxycytidine (dC), and
thymidine (T) in DNA and of adenosine (A), guanosine
35   (G), cytidine (C), and uridine (U) in RNA. Naturally
occurring modified nucleosides such as those containing

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2'-O-methylribosyl groups and those containing bases
such as N^4,N^4-dimethyladenine and N^7-methylguanidine are
found in messenger, transfer, and ribosomal RNA.
Internucleosidyl phosphodiester bonds link nucleosides

5   at the oxygen of the 3'-hydroxyl group of a D-ribose or
of a D-deoxyribose sugar moiety in one nucleoside to the
oxygen of a 5'-hydroxyl group in a D-ribose or D-
deoxyribose sugar moiety in another nucleoside in RNA
and in DNA, respectively.   Separate chains of nucleic

10   acids interact with one another via hydrogen bonds
formed between complementary pairs of nucleosidyl purine
and pyrimidine bases: adenine with thymine and guanine
with cytosine in DNA, and adenine with uracil and
guanine with cytosine in RNA.   When the sequences of

15   bases in two separate oligonucleotide strands or in two
regions of a single strand are complementary to each
other, the complementary sequences can hybridize with
each other via hydrogen bonds between complementary base
pairs to form a right-handed double helix with a B-type

20   conformation, the two phosphate-ribose ester backbones
of which are antiparallel.

Structure I
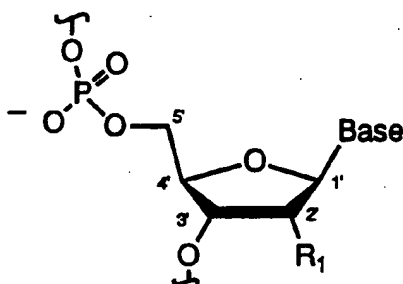
D-Enantiomers
D-DNA: R_1 = H
D-RNA: R_1 = OH

25

3

In naturally occurring DNA each nucleoside unit is
a D-enantiomer whose structure is defined by the
chirality of the D-deoxyribose ring which has
substituents at the 1'-(b), 3'-(a), and 4'-(b) positions

5   as represented schematically in Structure I wherein R_1
is H and "Base" represents an adenine, guanine, thymine
or cytosine moiety. In naturally occurring RNA each
nucleoside unit is also a D-enantiomer whose structure
is defined by the chirality of the D-ribose ring which

10   has substituents at the 1'-(b), 2'-(a), 3'-(a), and 4'-
(b) positions as represented schematically in Structure
I wherein R_1 is OH and "Base" represents an adenine,
guanine, uracil or cytosine moiety. In both naturally
occurring DNA and RNA, the phosphate diesters and the

15   bases do not comprise chiral centers.
As noted by P. S. Miller in Bioconjugate Chem.
1990, 1, 187-191, synthetically prepared
oligonucleotides composed of naturally configured D-
enantiomers have been used as primers for nucleic acid

20   polymerizing enzymes, as synthons in the construction of
artificial genes for the preparation of proteins by
recombinant DNA techniques, and as diagnostic probes to
detect and characterize cellular nucleic acid sequences.
In addition, synthetically prepared oligonucleotides

25   have been investigated for use in the control of gene
expression in living cells, and as therapeutic agents in
the inhibition of viral replication and in the treatment
of cancer. These applications rely on the sequence
specific complementary binding properties of the

30   synthetic oligonucleotides with natural D-enantiomeric
oligonucleotide target sequences.
Synthetically prepared oligonucleotides composed of
naturally configured D-enantiomers can be generated by a
variety of methods, the currently most useful of which

35   include solid phase synthesis via phosphoramidite
intermediates and solid phase synthesis via H-

phosphonate intermediates as described by E. Uhlmann and
A. Peyman in Chemical Reviews, 1990, 90, 544. In the
phosphoramidite method, a 5'-hydroxyl group of a growing
DNA oligomer chain of D-enantiomers having amide

5   protecting groups on the exocylic amine groups of the
bases therein and which is attached to a solid phase
support reacts with an activated D-nucleoside 5'-O-
dimethoxytrityl-3'-(2-cyanoethyl N,N-diisopropyl)-
phosphoramidite in the presence of 1H-tetrazole as a

10   catalyst. Commonly used protecting groups include the
benzoyl group for the protection of the exocyclic amino
groups of adenine and cytosine and the isobutyryl group
for the protection of the exocyclic amino group of
guanine. Any unreacted 5'-hydroxyl groups are capped

15   with acetate groups by reaction with acetic anhydride in
the presence of 4-N,N-dimethylaminopyridine. The
resulting phosphite is then oxidized with iodine to form
a phosphotriester. The 5'-O-dimethoxytrityl group is
removed under acid conditions using dichloroacetic acid,

20   and the reaction sequence is repeated using another
activated D-nucleoside 5'-O-dimethoxytrityl-3'-(2-
cyanoethyl N,N-diisopropyl) phosphoramidite. At the end
of the synthetic sequence, the oligonucleotide is freed
from amide protecting groups and cleaved from the

25   support by treatment with ammonium hydroxide. The
oligonucleotide is then purified or isolated by methods
such as precipitation, electrophoresis, or
chromatography.

        Many in vivo applications of synthetic D-

30   enantiomeric oligonucleotides are limited by the rapid
destruction of the oligomers by nucleases which are
encountered both extracellularly and intracellularly.
As reviewed by J. Goodchild in Bioconjugate Chem. 1990,
1, 165, strategies to introduce resistance to nuclease

35   activity included modification of the nucleoside bases,
and of the phosphate and ribose components of the

polymer backbone. All of these were done with the need to maintain the ability of the modified oligonucleotide to hybridize with a naturally occuring D-enantiomeric DNA or RNA target sequence or to bind with an enzyme
5   that uniquely recognizes the D-enantiomer.

The effects on the properties of D-oligonucleotides of a single modification of oligonucleoside chirality such as can be achieved through inversion at the 1'-ribose site to form an a-anomer, represented in
10   Structure II, have been studied by Bloch et al in Gene, 1988, 72, 349. These workers have found that alpha-anomeric DNA hybridizes with complementary beta-anomeric RNA, the naturally occurring enantiomeric oligomer, to form an a:b DNA:RNA hybrid, and that the hybrid is
15   nuclease resistant.

Structure II

α-Anomers
α-DNA: R₁ = H
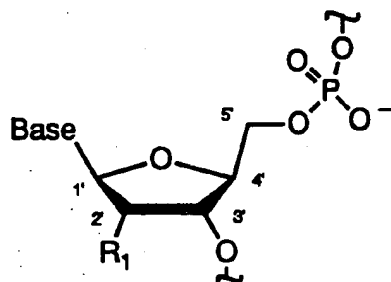α-RNA: R₁ = OH

20
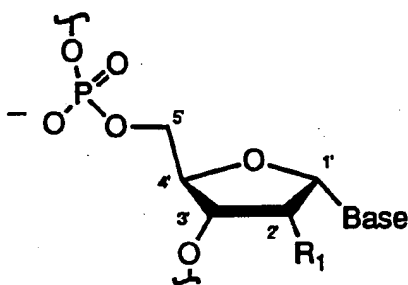
Thuong and Chassignol in Tetrahedron Letters, 1988, 29, 5905 reported the solid phase synthesis employing 2-cyanoethyl phosphoramidite synthons of oligo-a-deoxynucleotides containing a-d-T, a-d-C, and a-d-A
25   covalently linked at the 5'-phosphate to 2-methoxy-6-chloro-9-(5-hydroxypentylamino)acridine. Analogous oligo-a-deoxynucleotides without the acridine moiety

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were reported to be resistant to nucleases and to form
hybrids with natural D-RNA that were more stable than
those with b-deoxynucleotides. Morvan et al in Nucleic
Acid Research, 1988, 16, 833, reported the preparation

5   of oligo-a-deoxynucleotides containing a-d-T, a-d-C, a-
d-G, and a-d-A which formed double helix hybrids or
duplexes with complementary a and b strands with
parallel and antiparallel polarity, respectively. These
duplexes retained specific Watson-Crick base pairing

10   specificity, and the heteroduplexes were shown to belong
to the B-DNA family. They also showed higher thermal
stability when compared to the corresponding naturally
occurring bb-DNA duplexes. Again, however, the target
oligomer was a naturally occurring D-oligomer.

15   Complete inversion of all chiral sites of the D-
enantiomer in Structure I provides the mirror image,
non-naturally occurring L-enantiomer which is
represented in Structure III. In the absence of a
chiral environment or a chiral reagent such as an enzyme

20   which can distinguish between D- and L-enantiomers, the
L-enantiomer is identical in chemical reactivity to the
D-enantiomer. Urata et al in J. Am. Chem. Soc. 1991,
113, 8174 reported the synthesis (via the 2-cyanoethyl
phosphoramidite method) and properties of the self-

25   complementary L-DNA oligomer duplex, L-d(CGCGCG) and
compared them to those of the mirror image, natural D-
d(CGCGCG) oligomer. The unnatural all-L-duplex was
resistant to digestion by nuclease P-1 while the natural
all-D-duplex was rapidly degraded to the component D-

30   nucleotides and 5'-end deoxycytidine. As expected, both
the DD- and the LL-duplexes exhibited identical HPLC
retention times and their circular dichroism (CD)
spectra were mirror images of one another. In 0.1 M
NaCl, the D-enantiomer showed the CD profile of a

35   standard B form while the L-enantiomer exhibited the
same magnitude but opposite sign at all wavelengths.

This implied the existance of a mirror image B form
helix with left-handed double-helical conformation. In
4 M NaCl, the D-enantiomer showed the profile of a left-
handed Z form while the L-enantiomer exhibited the same

5   magnitude but opposite sign at all wavelengths
indicating the existance of a mirror image Z form with a
right-handed double helical conformation. The CD
spectra of both the all D-duplex and all L-duplex forms
exhibited the same B to Z conformational transition

10   (with opposite signs) as a function of salt
concentration as well as the same temperature dependence
under both low and high salt concentrations. It was
concluded that the D- and L-DNA have the same type and
strength of hydrogen bonding and base stacking

15   interactions, and that the structure of the L-DNA is the
exact mirror image of that of the D-DNA. This all L-
enantiomeric oligomer, being self-complementary, could
not be used to target either a separate natural D- or
unnatural L-enantiomer.

20

Structure III

L-Enantiomers
L-DNA: R_1 = H
L-RNA: R_1 = OH

25   Fujimori et al in Nucleic Acid Research, 1990, 22,
97 reported the synthesis of 9-(2-deoxy-b-L-*erythro*-

pentafuranosyl)-9H-purin-6-amine, L-dA, and the
hexameric L-DNA, L-d(AAAAAA). They observed L-d(AAAAAA)
to have resistance to bovine spleen phosphodiesterase
while D-d(AAAAAA) was completely degraded. They also
5   noted that the L-d(AAAAAA) formed a complex with a
natural D-RNA enantiomer, D-poly(U), which was weaker
relative to that formed between the enantiomeric natural
D-d(AAAAAA) and D-poly(U). No complex was formed
between the unnatural L-d(AAAAAA) and the natural DNA
10   enantiomer D-poly(T). These results suggested that
while L-enantiomeric DNA has the ability to distinguish
complementary RNA from DNA, the unnatural L-DNA:D-RNA
complexes are not nearly as strong as the natural
corresponding D-DNA:D-RNA complexes.
15   Although L-DNA has been shown to hybridize *in vitro*
with natural enantiomeric D-RNA and to be stable to
nuclease resistance, in practice such a duplex formation
with targeted D-RNA is not readily achievable *in vivo*
since it is necessary for the L-DNA to cross at least
20   one cell membrane to find the intact D-RNA.
Furthermore, natural D-RNA has a relatively short
lifetime in the presence of naturally occurring nuclease
enzymes.

25   Summary of the Invention
            The present invention is directed to a non-
radioactive targeting immunoreagent that comprises a
tumor antigen recognizing moiety, one or more L-
enantiomeric oligonucleotides comprising non-self-
30   associating L-enantiomeric oligonucleotide sequences,
and one or more linking groups.
            The present invention is also directed to a
radioactive targeting immunoreagent that comprises an L-
enantiomeric oligonucleotide comprising an L-
35   enantiomeric oligonucleotide sequence that is
complementary in sequence to and capable of

hybridization with one or more fragments of a non-self-
associating L-enantiomeric oligonucleotide sequence, one
or more chelating agents, one or more linking groups and
one or more radionuclides.

5   The present invention is also directed to
pharmaceutical compositions comprising one or more of
the above-described immunoreagents and a
pharmaceutically acceptable carrier.

           The present invention is further directed to
10   methods for treating and imaging disease sites such as
tumor sites in a patient. Said methods comprise
administration to the patient of an effective amount of
the above-described non-radioactive targeting
immunoreagent followed at an effective time interval by

15   an effective amount of the above-described radioactive
targeting immunoreagent.

           The present invention provides many advantages
compared to conventional targeting immunoreagents. For
example, the non-radioactive targeting immunoreagent can

20   accumulate at a tumor site in vivo while it is not
accumulated at normal tissue sites.

           The in vivo residence half life of the non-
radioactive targeting immunoreagent is long enough to
permit its accumulation at a tumor site.

25   The in vivo residence half life of the radioactive
targeting reagent is shorter than the residence half
life of the non-radioactive targeting immunoreagent.

           The portion of the radioactive targeting reagent
that does not hybridize to tumor associated non-

30   radioactive targeting reagent is rapidly cleared from
the patient.

           With respect to the same degree of modification of
a targeting immunoreagent by a directly labeled
radionuclide or a chelate containing a radionuclide, an

35   amplification of the number of radionuclides per site of

modification per targeting immunoreagent can be obtained.

A segment of the complementary sequenced L-enantiomeric oligonucleotide of the non-radioactive

5   targeting immunoreagent and a segment of the L-enantiomeric oligonucleotide of the radioactive targeting reagent can hybridize in vitro and in vivo.

The complementary sequenced L-enantiomeric oligonucleotides of the non-radioactive targeting

10   immunoreagent and the radioactive targeting reagent will not hybridize with isomeric, complementary sequenced, naturally occurring D-enantiomeric oligonucleotides.

The complementary sequenced L-enantiomeric oligonucleotides of the non-radioactive targeting

15   immunoreagent and the radioactive targeting reagent are stable to nuclease activity.

The complementary sequenced L-enantiomeric oligonucleotides of the non-radioactive targeting immunoreagent and the radioactive targeting reagent and

20   the hybrid formed between the complementary L-enantiomeric oligonucleotides will not bind to enzymes which are specific for binding to isomeric D-enantiomeric oligonucleotides.

The hybrid formed between complementary sequenced

25   L-enantiomeric oligonucleotides of the non-radioactive targeting immunoreagent and the radioactive targeting reagent is stable to nuclease activity.

The complementary sequenced L-enantiomeric oligonucleotides of the non-radioactive targeting

30   immunoreagent and the radioactive targeting reagent do not self hybridize.

The non-radioactive targeting immunoreagent and the radioactive targeting reagent can comprise a wide variety of spacing, linking, and chelating groups, L-

35   enantiomeric oligonucleotide sequences and radionuclides.

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The complementary sequenced L-enantiomeric
oligonucleotides of th non-radioactive targeting
immunoreagent can comprise L-enantiomeric
oligonucleotide sequences which can be tandemly linked
5   by spacing groups, wherein a segment of each L-
enantiomeric oligonucleotide sequence can hybridize with
a segment of the radioactive targeting reagent.

The complementary sequenced L-enantiomeric
oligonucleotides of the non-radioactive targeting
10   immunoreagent can be linked to an antibody by means of
either a 5'- or a 3'-substituent such as a 5'-amine or
3'-amine.

Reagents are provided that have a specificity for
tumors and a wide variety of compositions can be
15   prepared in accordance with the present invention.

A particular advantage of the present invention is
that L-enantiomeric oligonucleotide sequence lengths and
spacing groups can be selected such that on
hybridization of two complementary radioactive targeting
20   reagent L-enantiomeric oligonucleotide sequences to a
single L-enantiomeric oligonucleotide-containing strand
comprising adjacent, tandemly linked L-enantiomeric
oligonucleotide sequences of a non-radioactive targeting
immunoreagent, the proximal end groups of the sequences
25   of two such radioactive targeting moieties are
orthogonal to each other because of their relative
spacial configuration on the double stranded helix so
formed.

Other advantageous features of this invention will
30   become readily apparent upon reference to the following
description of the preferred embodiments.

Description of the Preferred Embodiments

This invention describes various novel
35   bioconjugates which possess utility in therapeutic and
diagnostic imaging compositions and methods. This

invention further describes novel methods of preparing bioconjugates by the attachment of various L-enantiomeric oligonucleotide sequences to chelating agents, preferably terpyridine containing chelating

5   agents, and to immunoreagents such as proteins, antibodies, and receptors.

In particular, this invention describes novel bioconjugates useful for sequential targeting and amplified delivery of novel radioactive immunoreagent

10   compositions which find particular utility in therapeutic and diagnostic imaging compositions and methods.

More particularly, this invention describes the preparation and use of targeting immunoreagents that

15   comprise a tumor antigen recognizing moiety, one or more L-enantiomeric oligonucleotides comprising non-self-associating L-enantiomeric oligonucleotide sequences, and one or more linking groups. These targeting immunoreagents react with a radioactive sequential

20   targeting reagent that comprises an L-enantiomeric oligonucleotide sequence that is complementary in sequence to and capable of hybridization with one or more fragments of the said non-self-associating L-enantiomeric oligonucleotide sequences, one or more

25   chelating agents, one or more linking groups, and having one or more radionuclides associated therewith. Most preferably, the L-enantiomeric nucleotides are L-enantiomers of natural D-deoxyribonucleotides.

In a preferred embodiment, the above-described

30   targeting immunoreagents form a compound that comprises moieties represented by the structure IV:
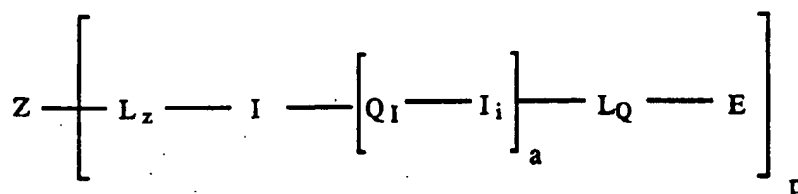
13

Structure IV

$$Z \left[L_z - I - \left[Q_I - I_i \right]_a - L_Q - E \right]_p$$

wherein:

Z is the residue of an immunoreactive protein;

L_z and L_Q are independently a chemical bond or a linking group;

I is an L-enantiomeric oligonucleotide comprising a contiguous sequence of from 12 to about 50 L-enantiomeric nucleotide units wherein said contiguous sequence contains one or more members of a family of homologous contiguous sequences, the individual homologs of said family comprising from 12 to about 30 L-enantiomeric nucleotide units, and provided that contiguous sequences of six or more L-enantiomeric nucleotide units of said L-enantiomeric oligonucleotide do not hybridize with any other contiguous sequences of six or more contiguous L-enantiomeric nucleotide units anywhere in structure IV;

Q_I is a spacing group;

a is 0 or an integer from 1 to about 6;

I_i is an L-enantiomeric oligonucleotide comprising a contiguous sequence of from 12 to about 50 L-enantiomeric nucleotide units, a contiguous sequence therein comprising a portion of I;

E is an end capping group; and

p is an integer from 1 to about 10.

More preferably, a is an integer from 1 to about 6.

In another preferred embodiment, the above-described targeting reagent comprises moieties represented by the structure V:

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described targeting reagent comprises moieties represented by the structure V:

<div align="center">Structure V</div>

$$W_1 - L_1 - cI - \left[Q_{cI} - L_2 \right]_b - W_2$$

$$\left[M_1 \right]_x \qquad \left[\begin{array}{c} L_3 \\ | \\ W_3 \\ \downarrow \\ \left[M_3 \right]_y \end{array} \right]_w \qquad \left[M_2 \right]_z$$
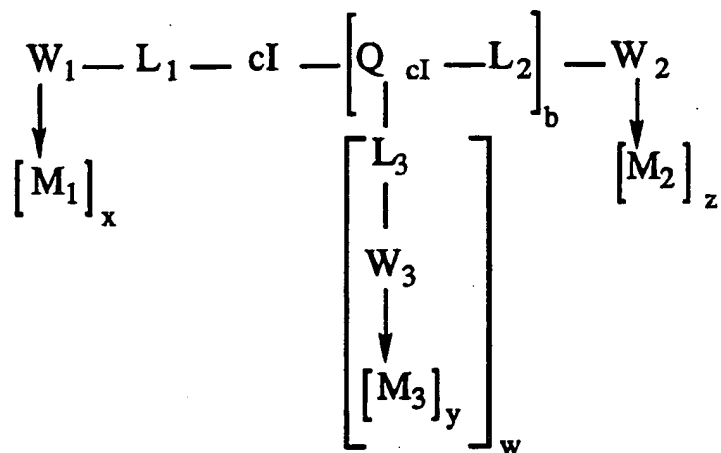
5 wherein:

cI is a contiguous sequence of from 12 to about 50 L-enantiomeric nucleotide units wherein said contiguous sequence contains one or more members of a family of

10 homologous contiguous sequences, the individual homologs of said family comprising from 12 to about 30 L-enantiomeric nucleotide units, where the nucleotide sequences of said homologs are complementary to the nucleotide sequences of members of the set of L-

15 enantiomeric oligonucleotides in a co-administerable targeting immunoreagent, and where contiguous sequences of six or more L-enantiomeric nucleotide units of said complementary L-enantiomeric oligonucleotide do not hybridize with any other contiguous sequences of six or

20 more contiguous L-enantiomeric nucleotide units anywhere in structure V;

Q_{cI} is a spacing group;

L_1, L_2, and L_3 are independently a chemical bond or a linking group;

25 W_1, W_2, and W_3 are each a residue of a chelating group;

<div align="center">15</div>

M_1, M_2, and M_3 comprise elements with oxidation states equal to or greater than +1, and at least one of M_1, M_2 and M_3 is a radionuclide;

x, y, and z are independently zero or one provided that at least one of x, y, or z is one; and

w and b are independently zero or an integer from 1 to about 4.

As used herein, an L-enantiomeric nucleotide unit is defined as the mirror image of the naturally occurring, isomeric D-enantiomer nucleotide unit; an L-enantiomeric nucleoside is defined as the mirror image of the naturally occurring, isomeric D-enantiomeric nucleoside; and an L-enantiomeric oligonucleotide sequence is defined as the mirror image of the naturally occurring, isomeric D-enantiomeric oligonucleotide sequence.

Non-limiting examples of L-enantiomeric nucleosides are set forth below.

1
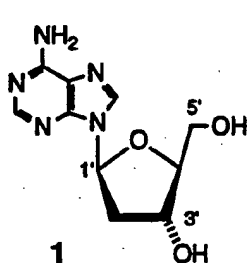
2-deoxy-L-adenosine, L-dA

2

2-deoxy-L-guanosine, L-dG

3

2-deoxy-L-uridine, L-dU

4
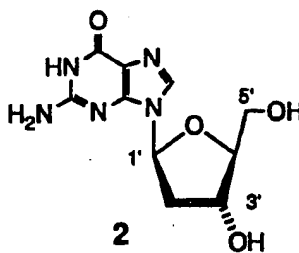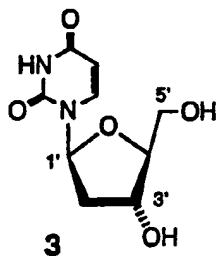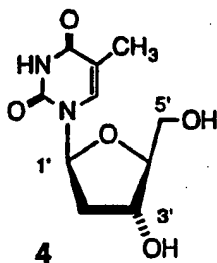
2-deoxy-L-thymidine, L-dT

5

2-deoxy-L-cytidine, L-dC

16
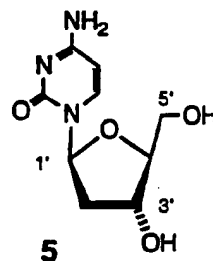
Methods of synthesis of L-enantiomeric
oligonucleotides from L-enantiomeric nucleotide
intermediates are identical to those used in the
5   synthesis of mirror image D-enantiomeric
oligonucleotides from isomeric D-enantiomeric nucleotide
intermediates. Examples of such methods include those
discussed in "Chemistry of Nucleosides and Nucleotides",
edited by Leroy B. Townsend, Plenum Press, N.Y., 1988.
10   The synthesis of L-enantiomeric oligonucleotides by such
methods requires the use of L-enantiomeric reagents such
as L-enantiomeric nucleoside and nucleotide derivatives.
The preparations of various L-enantiomeric nucleosides
are well documented by Robins, M. et al (1970), J. Org.
15   Chem., 35, p636-639; Holy, A. (1972), Coll. Czechoslov.
Chem. Commun., 37, p4072-4087; Visser, G. et al (1986),
Rec. Travaux Chim. Pay-Bas, 105/12 p528-537; Anderson,
D. et al (1984), Nucleosides and Nucleotides, 3/5,
p499-512; Uhlmann, E. et al (1990), Chem. Review,
20   (1990), 90/4 p543-584; Smejkal, I. et al (1964) Coll.
Czecholsov.Chem. Commun., 29, p2809-2813, and others.

Of the methods known in the art for the synthesis
of oligonucleotides, a preferred method of synthesis of
L-enantiomeric oligonucleotides of this invention
25   comprises solid phase synthesis utilizing L-enantiomeric
nucleotide phosphoramidite intermediates which contain
blocking groups on the hydroxyl groups therein. The
blocking of a 5'-hydroxyl group in a ribonucleoside
moiety and in a deoxyribonucleoside moiety is
30   preferrably done with an acid labile trityl group such
as, for example, a monomethoxytrityl group (sometimes
hereafter referred to as an MMT group) or a
dimethoxytrityl group (sometimes hereafter referred to
as a DMT group) employing the respective trityl
35   chlorides as reagents. The deblocking of such a trityl-
blocked 5'-hydroxyl group is preferably done with an

acid such as, for example, acetic acid in water, a
chloroacetic acid in a solvent such as dichloromethane,
or benzenesulfonic in a solvent such as chloroform or
methanol as a reagent. The blocking of a 2'-hydroxyl

5   group in a ribonucleic acid moiety is preferably done
with a silyl chloride reagent such as, for example, t-
butyldimethylchlorosilane which reacts with a ribosyl
2'-hydroxyl group to form a t-butyldimethylsilyl ether.
The deblocking of such a t-butyldimethylsilyl ether can

10   be achieved by treatment with, for example, sodium
hydroxide dissolved in a solvent such as, for example,
methanol.

            Preferred L-enantiomeric nucleotides are L-2-
deoxyribonucleotides, and preferred L-enantiomeric

15   oligonucleotides are oligo-L-2-deoxyribonucleotides.
Derivatives of L-enantiomeric deoxyribonucleotides that
are useful in the synthesis of the L-enantiomeric oligo-
L-deoxyribonucleotides of this invention are L-
enantiomeric deoxyribonucleosides such as, for example,

20   derivatives of compounds 1 to 5 which have the 5'-OH in
each blocked, for example, with a dimethoxytrityl (DMT)
group, each of which is activated at the 3'-OH for
phosphate bond formation in the oligonucleotide, for
example, by treatment with 2-cyanoethyl-N,N-

25   diisopropylphosphoryl chloride to produce the respective
3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite
intermediates. These are utilized for the synthesis of
oligo-L-deoxyribonucleotide moieties, for example, on a
solid phase, automated DNA synthesizer.

30   A preferred method of preparing a blocked 2'-deoxy-
L-adenosine 3'-O-phosphoramidite derivative, A10, that
is suitable for use in the synthesis of the L-
enantiomeric oligo-L-deoxyribonucleotide materials of
this invention is outlined in Scheme 1. 1-O-Methyl-3,5-

35   di-O-p-toluyl-2-deoxy-L-erythro-pentofuranose (A2 in
Scheme 1) is prepared from L-arabinal (A1) by treatment

with p-toluoyl chloride and methanol according to the
method described by Smejkal, I. et al (1964), Coll.
Czechoslov. Chem. Commun., 29, p2809-2813. Fusion of A2
and 2,6-dichloropurine (A3) provides 2,6-dichloro-9-
5   (3,5-di-0-p-toluyl-2-deoxy-alpha-L-erythro-
pentofuranosyl)purine (A4). Treatment of A4 with
methanol and ammonia selectively aminates the purine at
position 6 and concurrently deblocks the acylated
hydroxyl groups at the 3'- and 5'-positions. The
10   remaining chloride is removed by hydrogenolysis to
afford the desired L-nucleoside, L-dA, (A6). The
exocyclic amine of A6 is then blocked by diacylation
with benzoyl chloride to form A7 which contains two
benzoate esters in addition to the blocked amine. The
15   benzoate esters are removed by saponification with
sodium hydroxide in water to form A8. This diol is then
treated with dimethoxytrityl chloride (available from
Aldrich Chemical Company) to form A9 which has a DMT
ether at the 5'-position. A9 is then treated with 2-
20   cyanoethyl N,N-diisopropylchlorophosphoramidite
(available from Aldrich Chemical Company) to provide
A10, the desired diprotected 5'-O-dimethoxytrityl-2'-
deoxy-L-adenosine 3'-O-2-cyanoethylphosphoramidite. A10
is used as an intermediate in the synthesis of L-
25   oligonucleotide sequences of the compositions of
Structure IV and Structure V of this invention.
Production of such sequences can be done, for example,
using an automated oligonucleotide synthesizer using
procedures described by the manufacturer for the
30   synthesis and purification of isomeric D-
oligonucleotides.

Scheme 1 Preparation of a Blocked L-deoxyadenosine phosphoramidite
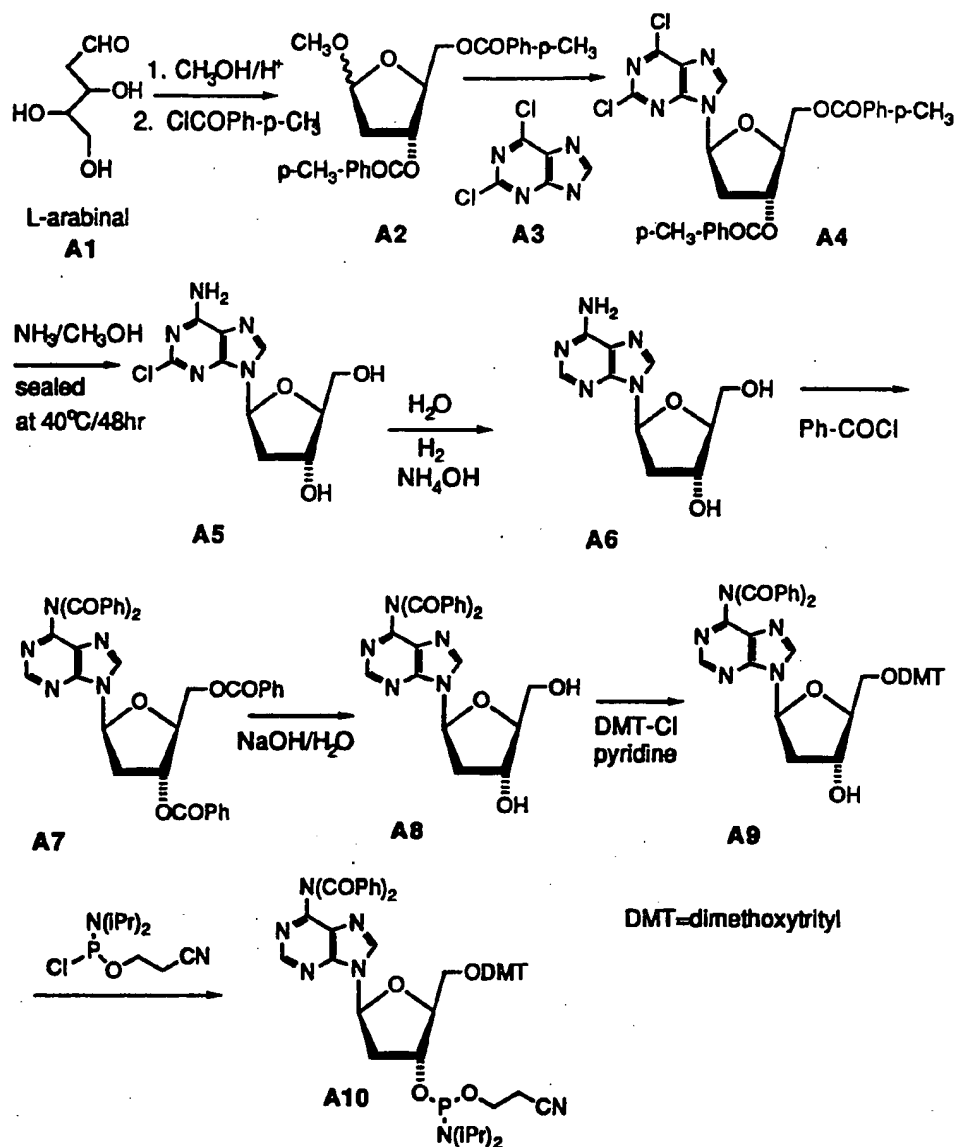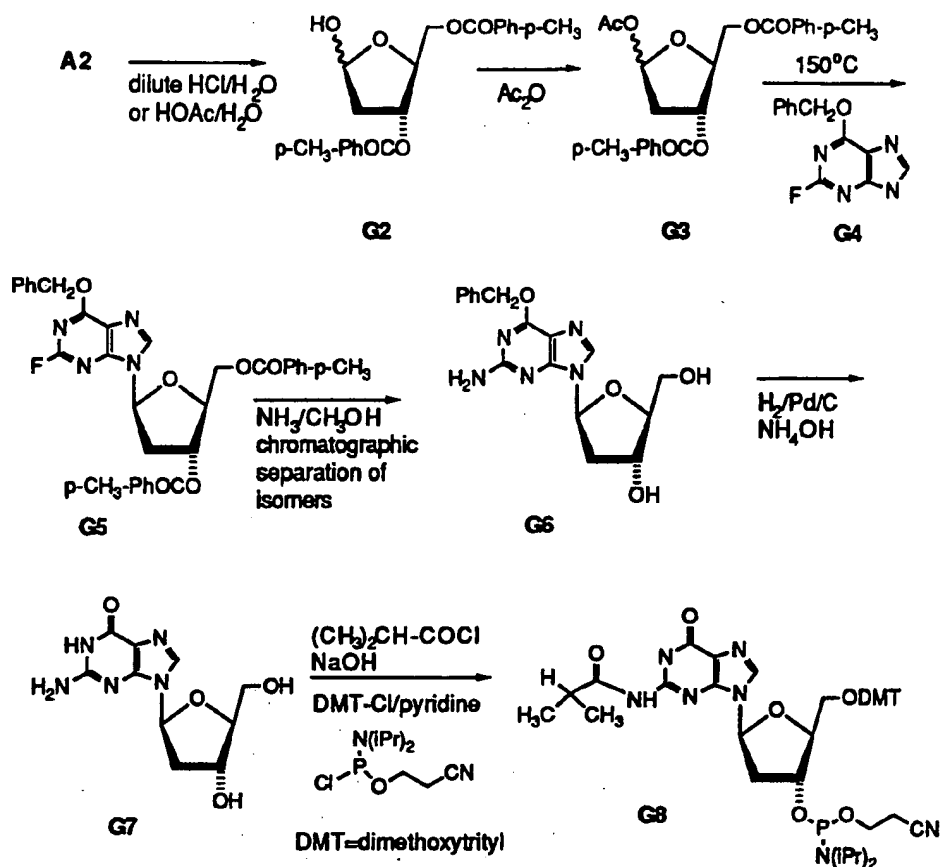

A preferred method of preparing a blocked 2'-deoxy-
L-guanosine 3'-O-phosphoramidite derivative, G8, that is
suitable for use in the synthesis of the L-enantiomeric
oligo-L-deoxyribonucleotide materials of this invention
is outlined in Scheme 2. 1-O-Methyl-3,5-di-O-p-toluyl-
2-deoxy-L-erythro-pentofuranose (A2 in Scheme 1) is
prepared from L-arabinal (A1) by treatment with p-

toluoyl chloride and methanol according to the method
described by Smejkal, I. et al (1964), Coll. Czechoslov.
Chem. Commun., 29, p2809-2813. The methyl acetal (A2)
is hydrolyzed in dilute hydrochloric acid to provide the

5   hemiacetal (G2) which is acylated with acetic anhydride
to form 1-acetyl 3,5-di-O-p-toluyl-beta-L-erythro-
pentofuranose (G3). Fusion of G3 and 2-fluoro-6-
benzyloxypurine (G4) provides G5 which is then treated
with alcoholic ammonia to provide the desired product

10   (G6) which is purified by chromatography. The 6-benzyl
protecting group of G6 is removed by hydrogenolysis with
palladium on carbon in ammonium hydroxide to yield 2-
amino-9-(2-deoxy-L-erythro-pentofuranosyl)purin-6-one
(G7; L-dG). The exocyclic 2-amino group of G7 is then

15   protected by acylation with isobutyryl chloride, the 5'-
hydroxyl group is protected as a DMT ether using
dimethoxytrityl chloride, and the 3'-hydroxyl is treated
with 2-cyanoethyl N,N-diisopropylchlorophosphoramidite
to provide G8, the desired diprotected 5'-O-

20   dimethoxytrityl-2'-deoxy-L-guanosine 3'-O-2-
cyanoethylphosphoramidite. G8 is used as an
intermediate in the synthesis of L-oligonucleotide
sequences of the compositions of Structure IV and
Structure V of this invention. Production of such

25   sequences can be done, for example, using an automated
oligonucleotide synthesizer using procedures described
by the manufacturer for the synthesis and purification
of isomeric D-oligonucleotides.

Scheme 2: Preparation of a blocked L-deoxyguanosine phosphoramidite

A preferred method of preparing a blocked 2'-deoxy-
L-uridine 3'-O-phosphoramidite derivative, U7, that is
suitable for use in the synthesis of the L-enantiomeric
oligo-L-deoxyribonucleotide materials of this invention
is outlined in Scheme 3. The reaction of L-arabinose
(U1) with cyanamide yields 2'-amino-1,2-oxazoline (U2)
which upon treatment with methyl propiolate (U3) affords
anhydro-L-uridine (U4). The anhydro derivative U4 is
opened with HBr to give the 2'-bromo-nucleoside (U5),
which upon catalytic hydrogenation affords 2-deoxy-L-
uridine (U6). The 5'-hydroxyl group of U6 is protected
as a DMT ether using dimethoxytrityl chloride, and the
3'-hydroxyl is treated with 2-cyanoethyl N,N-

diisopropylchlorophosphoramidite to provide U7, the
desired protected 5'-O-dimethoxytrityl-2'-deoxy-L-
uridine 3'-O-2-cyanoethylphosphoramidite. U7 is used as
an intermediate in the synthesis of L-oligonucleotide
5   sequences of the compositions of Structure IV and
Structure V of this invention. Production of such
sequences can be done, for example, using an automated
oligonucleotide synthesizer using procedures described
by the manufacturer for the synthesis and purification
10 of isomeric D-oligonucleotides.

Scheme 3: Preparation of a Blocked L-deoxyuridine phosphoramidite
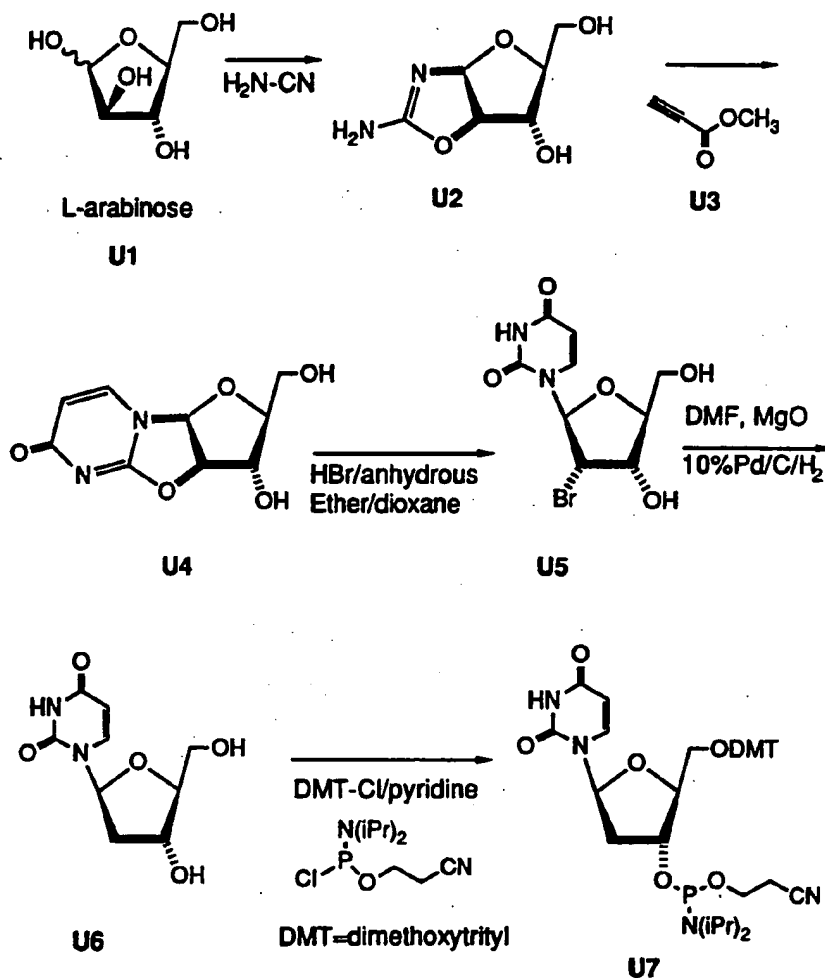
A preferred method of preparing a blocked L-
thymidine 3'-O-phosphoramidite derivative, T7, that is
suitable for use in the synthesis of the L-enantiomeric
5   oligo-L-deoxyribonucleotide materials of this invention
is outlined in Scheme 4. The synthetic method is
analogous to the preparation of the 2'-deoxy-L-uridine
3'-O-phosphoramidite derivative, U7, described in Scheme
3 using L-arabinose as a chiral starting material.
10   Accordingly, L-arabinose is treated with cyanamide to
provide 2'-amino-1,2-oxazoline (U2) which is then
reacted with methyl methacrylate with heating to yield
the anhydro-nucleoside (T4). T4 is opened with
anhydrous HBr to give 2-bromo-L-thymidine (T5). Upon
15   catalytic hydrogenation T5 affords L-thymidine (T6).
The 5'-hydroxyl group of T6 is protected as a DMT ether
using dimethoxytrityl chloride, and the 3'-hydroxyl is
treated with 2-cyanoethyl N,N-
diisopropylchlorophosphoramidite to provide T7, the
20   desired protected 5'-O-dimethoxytrityl-L-thymidine 3'-O-
2-cyanoethylphosphoramidite. T7 is used as an
intermediate in the synthesis of L-oligonucleotide
sequences of the compositions of Structure IV and
Structure V of this invention. Production of such
25   sequences can be done, for example, using an automated
oligonucleotide synthesizer using procedures described
by the manufacturer for the synthesis and purification
of isomeric D-oligonucleotides.

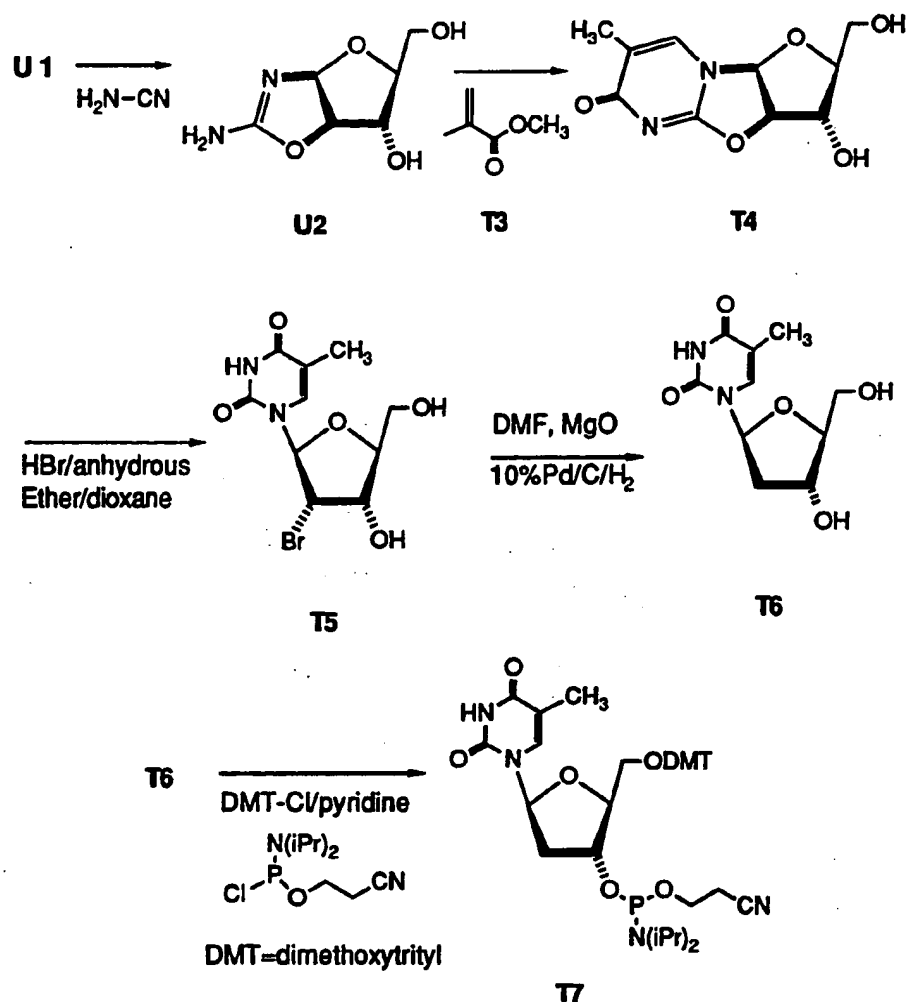## Scheme 4: Preparation of a Blocked L-thymidine phosphoramidite

A preferred method of preparing a blocked 2'-deoxy-L-cytidine 3'-O-phosphoramidite derivative, C7, that is suitable for use in the synthesis of the L-enantiomeric oligo-L-deoxyribonucleotide materials of this invention is outlined in Scheme 5. Thus, L-arabinal, A1, is treated with HCl and toluyl chloride to provide the 3,5-di-O-p-toluyl-2-deoxy-L-ribofuranosyl chloride (C2). Fusion of (C2) with 4-chloro-2-trimethylsiloxypyrimidine (C3) affords the 1-(3,5-di-O-p-toluyl-2-deoxy-L-ribofuranosyl)-4-chloropyrimidin-2-one (C4), which upon

treatment with ammonia in methanol yields 2-deoxy-L-
cytidine (C5). The exocyclic amine of C5 is protected
by treatment with benzoyl chloride, and the esters at
the 3'- and 5'-positions are saponified to regenerate C6

5 with free 3'- and 5'-hydroxyl groups. The 5'-hydroxyl
group of C6 is protected as a DMT ether using
dimethoxytrityl chloride, and the 3'-hydroxyl is treated
with 2-cyanoethyl N,N-diisopropylchlorophosphoramidite
to provide C7, the desired protected 5'-O-

10 dimethoxytrityl-2'-deoxy-L-cytidine 3'-O-2-
cyanoethylphosphoramidite. C7 is used as an
intermediate in the synthesis of L-oligonucleotide
sequences of the compositions of Structure IV and
Structure V of this invention. Production of such

15 sequences can be done, for example, using an automated
oligonucleotide synthesizer using procedures described
by the manufacturer for the synthesis and purification
of isomeric D-oligonucleotides.

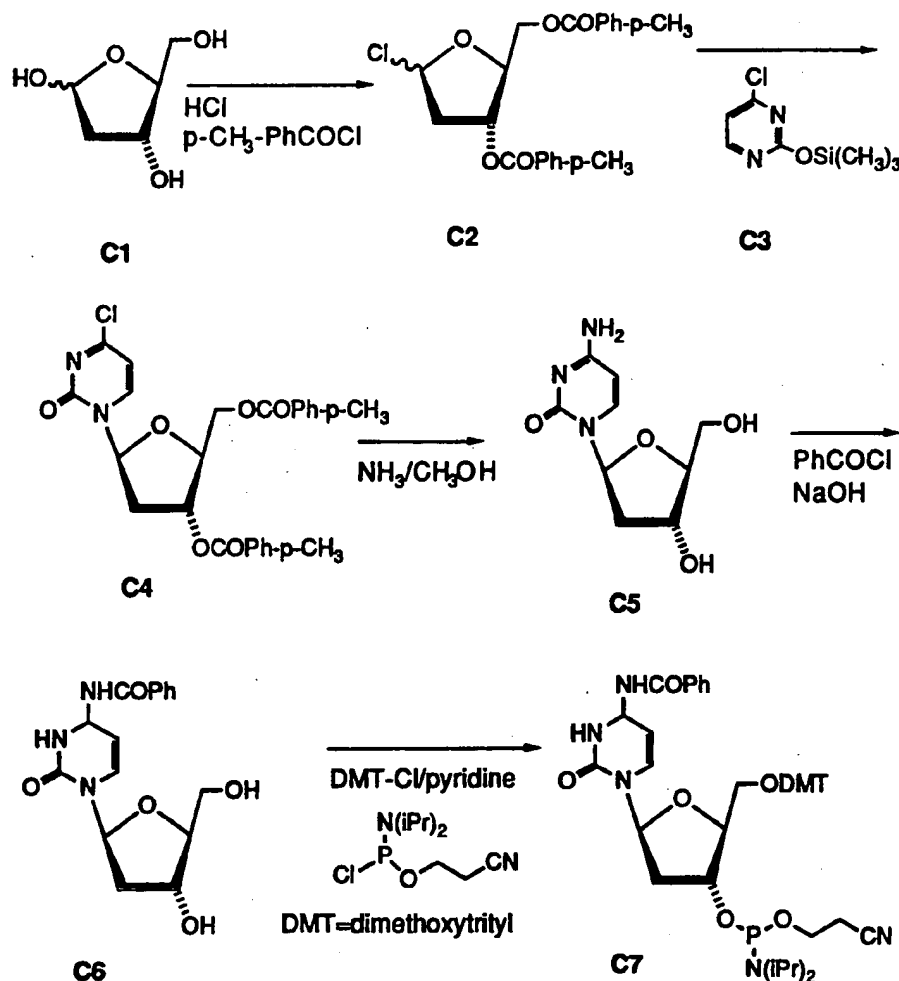**Scheme 5**: Preparation of a Blocked   L-deoxycytidine phosphoramidite

The 2'-deoxy-L-adenosine (A6) is sometimes hereinafter referred to as L-dA; the 2'-deoxy-L-guanosine (G7) is sometimes hereinafter referred to as L-dG; the 2'-deoxy-L-uridine (U6) is sometimes hereinafter referred to as L-dU; the L-thymidine (T6) is sometimes hereinafter referred to as L-T; and the 2'-deoxy-L-cytidine (C5) is sometimes hereinafter referred to as L-dC. Accordingly, as a definition of abbreviated nomenclature for the L-enantiomeric oligonucleotides of this invention, an irrelevant L-enantiomeric

oligonucleotide having a sequence containing, for
example, a 2'-deoxy-L-adenosine, L-dA, which is linked
by a phosphate diester group to a 2'-deoxy-L-guanosine,
L-dG, which in turn is linked by a phosphate diester

5   group to a 2'-deoxy-L-uridine, L-dU, which in turn is
linked by a phosphate diester group to an L-thymidine,
L-T, which in turn is linked by a phosphate diester
group to a 2'-deoxy-L-cytidine, L-dC, would be defined
and sometimes referred to as L-d(AGUTC). If the 5'-end

10   of this oligomer is attached to a group R_1 and the 3'-
end of this oligomer is attached to a group R_2, then the
oligomer would sometimes be referred to as R_1-5'-L-
d(AGUTC)-3'-R_2 or sometimes as 5'-R_1-L-d(AGUTC)-R_2-3'.
Conversely, if the 3'-end of this oligomer is attached

15   to a group R_1 and the 5'-end of this oligomer is
attached to a group R_2, then the oligomer would
sometimes be referred to as R_1-3'-L-d(AGUTC)-5'-R_2 or
sometimes as 3'-R_1-L-d(AGUTC)-R_2-5'. Reference is made
to the L-oligonucleotides of this invention and

20   particularly to the presently preferred oligonucleotides
which have the sequences as described hereinbelow using
this nomenclature.

The term "residue" is used herein in context with a
chemical entity. Said chemical entity comprises, for

25   example, a chelating group, or a linking group, or a
protein reactive group, or an immunoreactive group, or
an immunoreactive protein, or an antibody, or an
antibody fragment, or a cross-linking agent such as a
heterobifunctional cross-linking agent, or an L-

30   enantiomeric oligonucleotide, or a spacing group, or an
end capping group. The term "residue" is defined as
that portion of the chemical entity which exclusively
remains when one or more chemical bonds therein when
considered as an independent chemical entity, is

35   altered, modified, or replaced to comprise one or more
covalent bonds to one or more other chemical entities.

Thus, for example, in one aspect, a "residue of an L-
enantiomeric oligonucleotide" in the context of, for
example, I and I_i in Structure IV or of cI in Structure
V comprises an L-enantiomeric oligonucleotide modified

5   at least for divalent attachment to the residue of
another chemical entity, i.e., the residue of said L-
enantiomeric oligonucleotide comprises at least a
divalent L-enantiomeric oligonucleotidyl sequence. In
another aspect, for example, "the residue of a chelating

10   group" in the context of W_1, W_2 or W_3 of Structure V
comprises a chelating group which is at least
monovalently modified through attachment to the residue
of another chemical entity such as, for example, to the
residue of a linking group.

15   In Structure IV above, Z preferably is an antibody
or antibody fragment which recognizes and is specific
for a tumor associated antigen. In some embodiments,
the above-described protein can contain an
immunoreactive group covalently bonded thereto through a

20   chemical bond or a linking group derived from the
residue of a protein reactive group and the residue of a
reactive group on the protein. As used herein, the term
"immunoreactive protein" which can be abbreviated by
"IRP" also includes an organic compound which is capable

25   of covalently bonding to the protein and which is found
in a living organism or is useful in the diagnosis,
treatment or genetic engineering of cellular material or
living organisms, and which has a capacity for
interaction with another component which may be found in

30   biological fluids or associated with cells to be treated
such as tumor cells.

          The immunoreactive group can be selected from a
wide variety of naturally occurring or synthetically
prepared materials, including, but not limited to

35   enzymes, amino acids, peptides, polypeptides, proteins,
lipoproteins, glycoproteins, lipids, phospholipids,

hormones, growth factors, steroids, vitamins,
polysaccharides, viruses, protozoa, fungi, parasites,
rickettsia, molds, and components thereof, blood
components, tissue and organ components,

5   pharmaceuticals, haptens, lectins, toxins, nucleic acids
(including oligonucleotides), antibodies (monoclonal and
polyclonal), anti-antibodies, antibody fragments,
antigenic materials (including proteins and
carbohydrates), avidin and derivatives thereof, biotin

10   and derivatives thereof, and others known to one skilled
in the art. In addition, an immunoreactive group can be
any substance which when presented to an immunocompetent
host will result in the production of a specific
antibody capable of binding with that substance, or the

15   antibody so produced, which participates in an antigen-
antibody reaction.

Preferred immunoreactive groups are antibodies and
various immunoreactive fragments thereof, as long as
they contain at least one reactive site for reaction

20   with a protein reactive group as described herein on the
residue of the L-enantiomeric oligonucleotide or with
linking groups as described herein. That site can be
inherent to the immunoreactive species or it can be
introduced through appropriate chemical modification of

25   the immunoreactive species. In addition to antibodies
produced by the techniques outlined above, other
antibodies and proteins produced by the techniques of
molecular biology are specifically included.

Preferably, the immunoreactive group does not bind

30   to the residue of an L-enantiomeric oligonucleotide in
structure IV so as to inhibit the binding of the L-
enantiomeric oligonucleotide to a complementary
sequenced L-enantiomeric oligonucleotide of structure V.

As used herein, the term "antibody fragment" refers

35   to an immunoreactive material which comprises a residue
of an antibody, which antibody characteristically

exhibits an affinity for binding to an antigen. The term
affinity for binding to an antigen, as used herein,
refers to the thermodynamic expression of the strength
of interaction or binding between an antibody combining
site and an antigenic determinant and, thus, of the
stereochemical compatibility between them. As such, it
is the expression of the equilibrium or association
constant for the antibody-antigen interaction. The term
"affinity" as used herein also refers to the
thermodynamic expression of the strength of interaction
or binding between a ligand and a receptor and, thus, of
the stereochemical compatibility between them. As such,
it is the expression of the equilibrium or association
constant for the ligand-receptor interaction.

        With respect to the affinity of binding of an
antibody to an antigen, antibody fragments exhibit a
percentage of said affinity for binding to said antigen,
that percentage being in the range of 0.001 per cent to
1,000 per cent, preferably 0.01 per cent to 1,000 per
cent, more preferably 0.1 per cent to 1,000 per cent,
and most preferably 1.0 per cent to 100 per cent, of the
relative affinity of said antibody for binding to said
antigen.

        An antibody fragment can be produced from an
antibody by a chemical reaction comprising one or more
chemical bond cleaving reactions; by a chemical reaction
comprising of one or more chemical bond forming
reactions employing as reactants one or more chemical
components selected from a group comprising amino acids,
peptides, carbohydrates, linking groups as defined
herein, spacing groups as defined herein, protein
reactive groups as defined herein, and antibody
fragments such as are produced as described herein and
by a molecular biological process, a bacterial process,
or by a process comprising or resulting from the genetic
engineering of antibody genes.

An antibody fragment can be derived from an antibody by a chemical reaction comprising one or more of the following reactions:

(a) cleavage of one or more chemical bonds of which
5   an antibody is comprised, said bonds being selected from, for example, carbon-nitrogen bonds, sulfur-sulfur bonds, carbon-carbon bonds, carbon-sulfur bonds, and carbon-oxygen bonds, and wherein the method of said cleavage is selected from:

10   (i) a catalysed chemical reaction comprising the action of a biochemical catalyst such as an enzyme such as papain or pepsin which enzymes to those skilled in the art are known to produce antibody fragments commonly referred to as Fab and Fab'2, respectively;

15   (ii) a catalysed chemical reaction comprising the action of an electrophilic chemical catalyst such as a hydronium ion which, for example, favorably occurs at a pH equal to or less than 7;

(iii) a catalysed chemical reaction comprising the
20   action of a nucleophilic catalyst such as a hydroxide ion which, for example, favorably occurs at a pH equal to or greater than 7;

(iv) a chemical reaction comprising a substitution reaction employing a reagent such which is consumed in a
25   stoichiometric manner such as, for example, a substitution reaction at a sulfur atom of a disulfide bond by a reagent comprising a sulfhydryl group (comprising a -SH group) or an anionic sulfide group (comprising an -S$^-$ group in the form of a salt such as a
30   -S$^-$ Na$^+$ group);

(v) a chemical reaction comprising a reduction reaction such as, for example, the reduction of a disulfide bond; and

(vi) a chemical reaction comprising an oxidation
35   reaction such as the oxidation of a carbon-oxygen bond of a hydroxyl group or the oxidation of a carbon-carbon

bond of a vicinal diol group such as occurs in a
carbohydrate moiety; or

    (b) formation of one or more chemical bonds between
one or more reactants, such as formation of one or more
covalent bonds selected from, for example, carbon-
nitrogen bonds (such as, for example, amide bonds, amine
bonds, hydrazone bonds, imine bonds, and thiourea
bonds), sulfur-sulfur bonds such as disulfide bonds,
carbon-carbon bonds, carbon-sulfur bonds, and carbon-
oxygen bonds, and employing as reactants in said
chemical bond formation one or more reagents comprising
amino acids, peptides, carbohydrates, linking groups as
defined herein, spacing groups as defined herein,
protein reactive groups as defined herein, and antibody
fragments such as are produced as described in (a),
above; or

    (c) an antibody fragment can be derived by
formation of one or more non-covalent bonds between one
or more reactants. Such non-covalent bonds comprise
hydrophobic interactions such as occur in an aqueous
medium between chemical species that independently
comprise mutually accessible regions of low polarity
such as regions comprising aliphatic and carbocyclic
groups, and of hydrogen bond interactions such as occur
in the binding of an oligonucleotide with a
complementary oligonucleotide; or

    (d) an antibody fragment can be produced as a
result of the methods of molecular biology or by genetic
engineering of antibody genes, for example, in the
genetic engineering of a single chain immunoreactive
group or a Fv fragment.

    An antibody fragment can be produced as a result a
combination of one or more of the above methods.

    In certain embodiments, the immunoreactive group
can be an enzyme which has a reactive group for
attachment to the residue of an L-enantiomeric

oligonucleotide, I, by means of a linking group L$_Z$.
Representative enzymes include, but are not limited to,
aspartate aminotransaminase, alanine aminotransaminase,
lactate dehydrogenase, creatine phosphokinase, gamma
5   glutamyl transferase, alkaline acid phosphatase,
prostatic acid phosphatase, horseradish peroxidase and
various esterases.

        If desired, the immunoreactive group can be
modified or chemically altered to provide a reactive
10   group for use in the attachment to the residue of the L-
enantiomeric oligonucleotide, I, through a linking group
as described below by techniques known to those skilled
in the art. Such techniques include the use of linking
moieties and chemical modification such as described in
15   WO-A-89/02931 and WO-A-89/2932, which are directed to
modification of oligonucleotides, and U.S. Patent No.
4,719,182.

        Two highly preferred uses for the compositions of
this invention are for the diagnostic imaging of tumors
20   and the radiological treatment of tumors. Preferred
immunological groups therefore include antibodies to
tumor-associated antigens. An antibody is sometimes
hereinafter referred to as Ab.   Specific non-limiting
examples of antibodies include B72.3 and related
25   antibodies (described in U.S. Patent Nos. 4,522,918 and
4,612,282) which recognize colorectal tumors; 9.2.27 and
related anti-melanoma antibodies; D612 and related
antibodies which recognize colorectal tumors; UJ13A and
related antibodies which recognize small cell lung
30   carcinomas; NRLU-10, NRCO-02 and related antibodies
which recognize small cell lung carcinomas and
colorectal tumors (Pan-carcinoma); 7E11C5 and related
antibodies which recognize prostate tumors; CC49 and
related antibodies which recognize colorectal tumors;
35   TNT and related antibodies which recognize necrotic
tissue; PR1A3 and related antibodies which recognize

colon carcinoma; ING-1 and related antibodies, which are
described in International Patent Publication WO-A-
90/02569; B174, C174 and related antibodies which
recognize squamous cell carcinomas; B43 and related

5   antibodies which are reactive with certain lymphomas and
leukemias; and anti-HLB and related monoclonal
antibodies. An especially preferred antibody is ING-1.
Referring to structure IV again, L_Z and L_Q are
independently a chemical bond or the residue of a

10   linking group. The phrase "residue of a linking group"
as used herein refers to a moiety that remains, results,
or is derived from the reaction of a protein reactive
group with a reactive site on the protein. The phrase
"protein reactive group" as used herein refers to any

15   group which can react with a functional group typically
found on a protein. However, it is specifically
contemplated that a protein reactive groups can also
react with a functional group typically found on a non-
protein biomolecule. Thus a linking group useful in the

20   practice of this invention derives from a group which
can react with any biological molecule containing an
immunoreactive group, whether or not the biological
molecule is a protein, to form a linking group between
the immunoreactive group and the L-enantiomeric

25   oligonucleotide containing species as described below.
Preferred linking groups are derived from protein
reactive groups selected from but not limited to:
(1) A group that will react directly with amine,
alcohol, or sulfhydryl groups on the protein or

30   biological molecule containing the immunoreactive group,
for example, active halogen containing groups including,
for example, chloromethylphenyl groups and chloroacetyl
[$ClCH_2C(=O)-$] groups, activated 2-(leaving group
substituted)-ethylsulfonyl and ethylcarbonyl groups such

35   as 2-chloroethylsulfonyl and 2-chloroethylcarbonyl;
vinylsulfonyl; vinylcarbonyl; epoxy; isocyanato;

35

isothiocyanato; aldehyde; aziridine;
succinimidoxycarbonyl; activated acyl groups such as
carboxylic acid halides; mixed anhydrides and the like;
and other groups known to be useful in conventional

5   photographic gelatin hardening agents;

(2) A group that can react readily with modified
proteins or biological molecules containing the
immunoreactive group, i.e., proteins or biological
molecules containing the immunoreactive group modified

10   to contain reactive groups such as those mentioned in
(1) above, for example, by oxidation of a group such as
a hydroxyl group of a protein to an aldehyde or to a
carboxylic acid, in which case the "linking group" can
be derived from protein reactive groups selected from

15   amino, alkylamino, arylamino, hydrazino, alkylhydrazino,
arylhydrazino, carbazido, semicarbazido, thiocarbazido,
thiosemicarbazido, sulfhydryl, sulfhydrylalkyl,
sulfhydrylaryl, hydroxy, carboxy, carboxyalkyl and
carboxyaryl. The alkyl portions of said linking groups

20   can contain from 1 to about 20 carbon atoms. The aryl
portions of said linking groups can contain from about 6
to about 20 carbon atoms;

(3) A group that can be linked to the protein or
biological molecule containing the immunoreactive group,

25   or to the modified protein as noted in (1) and (2) above
by use of a crosslinking agent. The residues of certain
useful crosslinking agents, such as, for example,
homobifunctional and heterobifunctional gelatin
hardeners, bisepoxides, and bisisocyanates can become a

30   part of, i.e., a linking group in, the protein-(L-
enantiomeric oligonucleotide-containing species)
conjugate during the crosslinking reaction. Other
useful crosslinking agents, however, can facilitate the
crosslinking, for example, as consumable catalysts, and

35   are not present in the final conjugate. Examples of
such crosslinking agents are carbodiimide and

36

carbamoylonium crosslinking agents as disclosed in U.S.
Patent No. 4,421,847 and the ethers of U.S. Patent No.
4,877,724. With these crosslinking agents, one of the
reactants, such as the immunoreactive group, must have a

5   carboxyl group and the other, such as the L-enantiomeric
oligonucleotide-containing species, must have a reactive
amine, alcohol, or sulfhydryl group. In amide bond
formation, the crosslinking agent first reacts
selectively with the carboxyl group, then is split out

10   during reaction of the thus "activated" carboxyl group
with an amine to form an amide linkage between the
protein and L-enantiomeric oligonucleotide containing
species, thus covalently bonding the two moieties. An
advantage of this approach is that crosslinking of like

15   molecules, e.g., proteins with proteins or L-
enantiomeric oligonucleotide containing species with
themselves is avoided, whereas the reaction of, for
example, homo-bifunctional crosslinking agents is
nonselective and unwanted crosslinked molecules are

20   obtained.
       Preferred useful linking groups are derived from
various heterobifunctional cross-linking reagents such
as those listed in the Pierce Chemical Company
Immunotechnology Catalog - Protein Modification Section,

25   (1991 and 1992).
       Useful non-limiting examples of such reagents
include:

30   Sulfo-SMCC   Sulfosuccinimidyl 4-(N-
                           maleimidomethyl)cyclohexane-1-
                           carboxylate;

            Sulfo-SIAB   Sulfosuccinimidyl (4-
35   iodoacetyl)aminobenzoate;

Sulfo-SMPB   Sulfosuccinimidyl 4-(p-
                    maleimidophenyl)butyrate;

2-IT   2-Iminothiolane; and

5

SATA   N-Succinimidyl S-acetylthioacetate.

In addition to the foregoing description, the
linking groups, in whole or in part, can also comprise
10 and be derived from nucleotides and residues of
nucleotides, both naturally occurring and modified.
Particularly useful, non-limiting reagents for
incorporation of modified nucleotide moieties containing
reactive functional groups, such as amine and sulfhydryl
15 groups, into an L-enantiomeric oligonucleotide sequence
of this invention are commercially available from, for
example, Clonetech Laboratories Inc. (Palo Alto
California) and include Uni-Link AminoModifier (Catalog
5190), Biotin-ON phosphoramidite (Catalog # 5191), N-
20 MNT-C6-AminoModifier (Catalog # 5202), AminoModifier II
(Catalog # 5203), DMT-C6-3'Amine-ON (Catalog # 5222),
C6-ThiolModifier (Catalog # 5211), and the like. In one
aspect, linking groups of this invention are derived
from the reaction of a reactive functional group such as
25 an amine or sulfhydryl group as are available in the
above Clonetech reagents, one or more of which has been
incorporated into an L-enantiomeric oligonucleotide
sequence of this invention, with, for example, one or
more of the previously described protein reactive groups
30 such as heterobifunctional protein reactive groups, one
or more of which has been incorporated into an
immunoreagent of this invention.
    Referring to Structure IV again, I and I_1 each
independently comprise an L-enantiomeric oligonucleotide
35 of a contiguous sequence of from 12 to about 50 L-
enantiomeric nucleotide units wherein said contiguous

sequence contains one or more members of a family of
homologous contiguous sequences; wherein the individual
homologs of said family comprise from 12 to about 30 L-
enantiomeric nucleotide units; wherein the homologs of

5   said family of sequences, both individually or as a set
of homologous sequences being hereinafter sometimes
referred to as "the Sequence"; and wherein any
contiguous sequence of six or more L-enantiomeric
nucleotide units does not hybridize with any other

10   contiguous sequence of six or more contiguous L-
enantiomeric nucleotide units anywhere in structure IV.
Members of the set of homologous contiguous sequences
which comprise "the Sequence" can be found in both the
sequence I and the sequence I$_i$, and at least one such

15   sequence is common to both I and I$_i$.

        The L-enantiomeric oligonucleotide sequence of I
and I$_i$ in Structure IV can comprise L-DNA, L-RNA, purine
and pyrimidine base-modified L-DNA or L-RNA, backbone-
modified L-DNA or L-RNA such as methyl phosphonate or

20   thiophosphonate or carbohydrate modified L-DNA or L-RNA
analogs, whole or partially modified, or combinations
thereof as long as a complementary L-enantiomeric
oligonucleotide sequence incorporated into the
radioactive targeting moiety of Structure V described

25   below can hybridize to said L-enantiomeric
oligonucleotide sequence to form a hybrid which exhibits
a Tm (melting temperature) greater than about 37 ^{o}C.
Preferred L-enantiomeric oligonucleotides are non-base-
modified and non-backbone-modified L-DNA and L-RNA, more

30   preferred are L-DNA comprising L-dA, L-T, L-dG, L-dU and
L-dC L-enantiomeric nucleotide units. Currently
especially preferred L-enantiomeric oligonucleotides are
L-DNA comprising L-dA, L-T, L-dG, and L-dC L-
enantiomeric nucleotide units.

35   In a preferred embodiment, the L-enantiomeric
oligonucleotide sequ nce I and I$_i$ can comprise double

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stranded L-DNA or L-RNA. That is, the L-enantiomeric
oligonucleotide sequence may comprises complementary L-
DNA or L-RNA which forms a double helix molecule. The
complementary L-enantiomeric oligonucleotide sequence
5   incorporated into the radioactive targeting moiety,
composed of L-DNA or L-RNA, then hybridizes to one or
the other of the strands of the double stranded L-DNA or
L-RNA comprising I and I_i. In this way, the
complementary L-enantiomeric oligonucleotide sequence
10   incorporated into the radioactive targeting moiety
interacts with the duplex L-DNA or L-RNA of I and I_i in
such a way as to form triplex (triple helix) L-DNA,
triplex L-RNA, or a triplex L-DNA:L-RNA hybrid.

Preferred non-limiting examples of L-enantiomeric
15   oligonucleotide sequences comprising the "Sequence" are
shown below. The following sequences comprise a set of
homologous L-enantiomeric oligonucleotide sequences
which when considered individually or in any combination
comprise a set herein defined as the "Sequence":
20   (i)   L-d(TTATGGACGGAG) (SEQ ID NO:1);
(ii)   L-d(TTATGGACGGAGA) (SEQ ID NO:2);
(iii) L-d(TTATGGACGGAGAA) (SEQ ID NO:3);
(iv)   L-d(TTATGGACGGAGAAG) (SEQ ID NO:4);
(v)   L-d(TTATGGACGGAGAAGC) (SEQ ID NO:5);
25   (vi)   L-d(TTATGGACGGAGAAGCT) (SEQ ID NO:6);
(vii) L-d(TTATGGACGGAGAAGCTA) (SEQ ID NO:7); and
(viii) L-d(TTATGGACGGAGAAGCTAA) (SEQ ID NO:8).

Of course, sequence (viii) contains sequence (vii)
which contains sequence (vi) which contains sequence
30   (v), and so on. Thus, in structure IV, if I contains,
for example sequence (iii), and an I_i contains, for
example, sequence (v), then both I and I_i contain at
least sequence (iii). Another I_i in structure IV can
contain (viii), in which case it would also contain (i)
35   through (vii) as well as (viii). In this case, all
three sequences would contain at least (iii) [as well as

(i) and (ii)], and the two I₁'s would contain at least
(v) [as well as (i) through (iv)]. In this regard, an
L-enantiomeric oligonucleotide that comprises a
contiguous sequence of L-enantiomeric nucleotides, which
5   sequence being complementary to at least sequence (i),
would hybridize to all sequences (i) through (viii) as
would any member of a set of contiguous complementary
sequences, the individual members of which comprise the
sequence complementary to any of (i) through (viii).
10   Such a set of contiguous complementary sequences can
comprise cI as will be described below.

     Another set of preferred homologous L-enantiomeric
oligonucleotide sequences comprising the "Sequence" is:
       (ix)   L-d(CGGAGAAGCTAA) (SEQ ID NO:9);
15   (x)   L-d(ACGGAGAAGCTAA) (SEQ ID NO:10);
       (xi)   L-d(GACGGAGAAGCTAA) (SEQ ID NO:11);
      (xii)   L-d(GGACGGAGAAGCTAA) (SEQ ID NO:12);
     (xiii)   L-d(TGGACGGAGAAGCTAA) (SEQ ID NO:13);
      (xiv)   L-d(ATGGACGGAGAAGCTAA) (SEQ ID NO:14);
20   (xv)   L-d(TATGGACGGAGAAGCTAA) (SEQ ID NO:15); and
      (xvi)   L-d(TTATGGACGGAGAAGCTAA) (SEQ ID NO:8).

     An especially preferred sequence is: L-
d(TTATGGACGGAGAAGCTAA) (SEQ ID NO:8).

     Two or more of the L-enantiomeric oligonucleotide
25   sequences of this invention can be tandemly linked by
means of chemical bonds, by linking groups such as
described above, or by spacing groups as described
below. The sequential order of L-enantiomeric
nucleotides in the L-enantiomeric oligonucleotide
30   sequences of this invention can be from the 5' to the 3'
end or from the 3' to the 5' end. Attachment of the L-
enantiomeric oligonucleotide sequences of this invention
via linking groups as described above to the immune
reactive group as described above can be accomplished
35   via 3' or via 5' sites or via derivatives attached to 3'
or 5' sites of the L-enantiomeric oligonucleotide.

As discussed above, the "Sequence" may also be composed of a double stranded L-DNA or L-RNA. That is, the "Sequence" may consist of complementary L-enantiomeric oligonucleotides which non-covalently interact to form double stranded L-DNA or L-RNA. Attachment of this double stranded nucleic acid to the immune reactive group as described above can be accomplished via 3' or via 5' sites or via derivatives attached to 3' or 5' sites of the L-enantiomeric oligonucleotide.

Referring to structure IV again, Q_I is a spacing group which separates and links two or more L-enantiomeric oligonucleotide sequences of this invention. Q_I can comprise a linking group as defined above, alone, or in combination with an L-enantiomeric nucleotide or an L-enantiomeric oligonucleotide comprising 2 to about 20 L-enantiomeric nucleotide units, the sequence of which is not self-associating or such that contiguous sequences of six or more L-enantiomeric nucleotide units therein do not hybridize with any other contiguous sequences of six or more contiguous L-enantiomeric nucleotide units anywhere in structure IV. Q_I can also comprise a residue of an amino acid group, a peptide group, or a poly(alkylene oxide) group such as a poly(ethylene glycol) group. It is contemplated that each spacing group can be linked to from two to about six L-enantiomeric oligonucleotide sequences, at least two of which contain the Sequence of this invention. Preferably, the spacing group is linked to two L-enantiomeric oligonucleotide sequences each of which contains the Sequence of this invention. Preferably, the spacing group is an L-enantiomeric oligonucleotide sequence.

Non limiting examples of preferred spacing groups are L-enantiomeric oligonucleotides comprising the following sequences:

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L-d(ACT);

L-d(ACTC);

L-d(ACTCT);

L-d(CTC);

5   L-d(TCTC); and

L-d(CTCTC).

An especially preferred spacing group is an L-
enantiomeric oligonucleotide: L-d(ACTCTC).

Of course, the condition described above, i.e.,

10   that the L-enantiomeric oligonucleotide sequence of this
invention comprises a non-self associating sequence,
still applies when considering the selection of L-
enantiomeric oligonucleotide spacing groups linked in
combination with the L-enantiomeric oligonucleotide

15   Sequence groups.

In structure IV, a is from 0 to about 6, preferably
an integer from 1 to about 6, more preferably one to
about 4, and most preferably one or two.

In structure IV, p is an integer from 1 to 10,

20   preferably 1 to about 6, and more preferably 1 to 3. It
is also contemplated that mixtures of immunoreactive
proteins comprising mixtures of Z modified as defined in
structure IV together with Z not so modified will also
be useful in this invention. In this case, the bulk

25   mixture properties of "p" of such mixtures would
comprise fractional values from about zero to about 10.
Preferably, the bulk p values would be from about 0.1 to
about 10.0, more preferably from about 0.2 to about 5.0,
and most preferably from about 0.4 to about 3.

30   In structure IV, E is an end capping group. E is
preferably an L-enantiomeric nucleotide group or a group
of one or more D-oligonucleotides that is modified so as
to reduce or prevent the action of exonuclease enzymatic
activity on the D-enantiomeric oligonucleotide sequence

35   therein. E can be a 3'- or 5'-phosphate-linked ribose
group containing one or more substituents such as an

alkyl group of 1 to about 10 carbon atoms. E can be a
5'- or 3'-ether group such as an alkyl ether, an aryl
ether, an aralkyl ether, a substituted aryl ether or an
aralkyl ether wherein the alkyl groups contain from 1 to
5   about 10 carbon atoms and the aryl groups contain from 6
to 10 carbon atoms, and wherein the alkyl or aryl groups
may contain oxygen, nitrogen or sulfur atoms or be
substituted by alkyl or aryl groups containing oxygen,
nitrogen or sulfur atoms. E can be a 5'-O- or 3'-O-
10   phosphate ester group such as an alkyl ester, an aryl
ester, an aralkyl ester, a substituted aryl ester or an
aralkyl ester wherein the alkyl groups contain from 1 to
about 10 carbon atoms and the aryl groups contain from 6
to 10 carbon atoms, and wherein the alkyl or aryl groups
15   may contain oxygen, nitrogen or sulfur atoms or be
substituted by alkyl or aryl groups containing oxygen,
nitrogen or sulfur atoms. E can be a poly(alkylene
oxidyl) group on the ribosyl moiety, preferably at the
5'- or 3'- position, either as an ether group or linked
20   by a phosphate ester to the 5'- or 3'-oxygen of the L-
ribosyl group. The poly(alkylene oxidyl) group can be,
for example, a poly(ethylene oxidyl) or poly(propylene
oxidyl) or a poly(propylene oxidyl-co-ethylene oxidyl)
group, each polymer containing from 2 to about 100
25   repeating units. A phosphate ester comprising such
entities is also useful, as well as a phosphate ester or
modified ribose comprising elements of a suitable
linking group as defined above. E can also comprise a
Z or it can be attached to Q_I by elements of L_Z as
30   defined above to form a cyclic structure. E can also
comprise compounds with a two carbon-one nitrogen atom
internucleoside linkage. Preferably, E comprises a
poly(alkylene glycol) phosphate diester. With respect
to E, the poly(alkylene glycol) moiety can have from 2
35   to about 100 repeating units. Preferably, the
poly(alkylene glycol) is a poly(ethylene glycol). A

currently preferred poly(alkylene glycol) phosphate
diester is a tetra(ethylene glycol) phosphate diester,
hereinafter sometimes referred to as a "Teg" or "Teg
unit". Such poly(alkylene glycol) phosphate diesters

5   can be linked in tandem to each other to form a dimer
phosphate ester sequence, a trimer sequence, a tetramer
sequence, and so forth. One or two such units is
preferred. Such units can also be attached to residues
of Q_I, L_Z, L_Q, I, I_i or Z described above. A preferred

10   end group E comprises a Teg unit linked by a phosphate
ester bond to an L-enantiomeric nucleotide such as T.
Other preferred end capping units for an L-DNA sequence
comprise a residue of L-dA, L-dG, L-T, L-dC, and L-dU or
an oligonucleotide sequence comprised therefrom.

15   In the context of this invention, the term
"modified nucleotide moiety" is intended to mean a
chemical entity which comprises one or more chemical
groups that are analogous to one or more portions of a
naturally occurring D-enantiomeric nucleotide or of a

20   residue of a naturally occurring D-enantiomeric
nucleotide. A "modified D-enantiomeric nucleotide
moiety" comprises that chemical entity which exclusively
remains when one or more chemical bonds, of which said
naturally occurring D-enantiomeric nucleotide is

25   otherwise comprised when considered as an independent
chemical entity, is altered, modified, or replaced to
comprise one or more covalent bonds to one or more other
chemical entities, or comprises that chemical entity
which exclusively remains after removal or deletion of a

30   portion, such as, for example, a purine or pyrimidine
base portion, a hydroxyl portion, a ribose portion, and
the like or combinations thereof, of the naturally
occurring D-enantiomeric nucleotide in one location
simultaneously with said replacement of another portion

35   of said L-enantiomeric nucleotide. Particularly useful,
non-limiting examples of modified D-enantiomeric

nucleotide moieties comprise reactive functional groups,
such as amine and sulfhydryl groups. They can be
commercially available such as, for example, those
modified D-enantiomeric nucleotide moieties and

5   precursors thereto which are available from Clonetech
Laboratories Inc. (Palo Alto, California). Said
modified D-enantiomeric nucleotide moieties and
precursors thereto include Uni-Link AminoModifier
(Catalog #5190), Biotin-ON phosphoramidite (Catalog

10   #5191), N-MNT-C6-AminoModifier (Catalog #5202),
AminoModifier II (Catalog #5203), DMT-C6-3'Amine-ON
(Catalog #5222), C6-ThiolModifier (Catalog #5211), and
the like. One or more of said moieties can be
incorporated into an L-enantiomeric oligonucleotide

15   sequence comprising this invention. In one aspect,
linking groups of this invention are derived from the.
reaction of a reactive functional group such as an amine
or sulfhydryl group as are available in the above
Clonetech reagents, one or more of which has been

20   incorporated into an L-enantiomeric oligonucleotide
sequence of this invention, with, for example, one or
more of the previously described protein reactive groups
such as heterobifunctional protein reactive groups, one
or more of which has been incorporated into an

25   immunoreagent of this invention.
        A "modified D-enantiomeric nucleotide moiety" can
comprise a D-enantiomeric nucleotide moiety that is
modified so as to reduce or prevent the action of
exonuclease enzymatic activity on the D-enantiomeric

30   oligonucleotide sequence. It can be a 3'- or 5'-
phosphate linked ribose or a 3'- or 5'-phosphate linked
2'-deoxyribose group containing one or more substituents
such as an alkyl group of 1 to about 10 carbon atoms, or
an ether group such as alkyl or aryl or aralkyl or

35   substituted aryl or aralkyl ether wherein the alkyl
groups contain from 1 to about 10 carbon atoms and such

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alkyl or aryl groups may contain or be substituted by
substituents containing oxygen, nitrogen or sulfur
atoms, or a poly(alkylene oxidyl) group, preferably at
the 5'- or 3'-ribose position, respectively, or

5   elsewhere on the ribose group, which substituent will
reduce or prevent the action of exonuclease enzymatic
activity. A "modified D-enantiomeric nucleotide moiety"
comprising a phosphate ester comprising said
substituents is also useful, as well as a phosphate

10   ester or modified ribose comprising elements of a
suitable linking group as defined above. A "modified D-
enantiomeric nucleotide moiety" can also comprise a
residue of Z or it can be attached to Q_I by elements of
L_Z as defined above to form a cyclic structure. A

15   "modified D-enantiomeric nucleotide moiety" can also
comprise compounds with a two carbon-one nitrogen atom
internucleoside linkage.

Preferably, a "modified D-enantiomeric nucleotide
moiety" comprises a poly(alkylene glycol) phosphate

20   diester. With respect to said "modified D-enantiomeric
nucleotide moiety", the poly(alkylene glycol) moiety can
have from 2 to about 100 repeating units. Preferably,
the poly(alkylene glycol) is a poly(ethylene glycol). A
currently preferred poly(alkylene glycol) phosphate

25   diester is a tetra(ethylene glycol) phosphate diester,
hereinafter sometimes referred to as a "Teg" or "Teg
unit". Such poly(alkylene glycol) phosphate diesters
can be linked in tandem to each other to form a dimer
phosphate ester sequence, a trimer sequence, a tetramer

30   sequence, and so forth. One or two such units is
preferred. Such units can also be attached to residues
of Q_I, L_Z, L_Q, I, I_i or Z described herein. A preferred
"modified D-enantiomeric nucleotide moiety" comprises a
Teg unit linked by a phosphate ester bond to a D-

35   enantiomeric nucleotide such as L-T.

Referring to the radioactive targeting
immunoreagent described in Structure V, preferred non-
limiting examples of a set of L-enantiomeric
oligonucleotides, cI, which are complementary to the

5   members of the set of L-enantiomeric oligonucleotides
comprising the "Sequence" of I in structure IV include
the L-DNA L-enantiomeric oligonucleotides:

    (xvii)   L-d(TTAGCTTCTCCG) (SEQ ID NO:16);

    (xviii) L-d(TTAGCTTCTCCGT) (SEQ ID NO:17);

10   (xix)   L-d(TTAGCTTCTCCGTC) (SEQ ID NO:18);

    (xx)   L-d(TTAGCTTCTCCGTCC) (SEQ ID NO:19);

    (xxi)   L-d(TTAGCTTCTCCGTCCA) (SEQ ID NO:20);

    (xxii)   L-d(TTAGCTTCTCCGTCCAT) (SEQ ID NO:21);

    (xxiii)   L-d(TTAGCTTCTCCGTCCATA) (SEQ ID NO:22);

15   and

    (xxiv)   L-d(TTAGCTTCTCCGTCCATAA) (SEQ ID NO:23)

Another set of preferred homologous L-enantiomeric
oligonucleotide sequences which can comprise a set
complementary to the "Sequence" of structure IV is:

20   (xxv)   L-d(CTCCGTCCATAA) (SEQ ID NO:24);

    (xxvi)   L-d(TCTCCGTCCATAA) (SEQ ID NO:25);

    (xxvii)   L-d(TTCTCCGTCCATAA) (SEQ ID NO:26);

    (xxviii)   L-d(CTTCTCCGTCCATAA) (SEQ ID NO:27);

    (xxix)   L-d(GCTTCTCCGTCCATAA) (SEQ ID NO:28);

25   (xxx)   L-d(AGCTTCTCCGTCCATAA) (SEQ ID NO:29);

    (xxxi)   L-d(TAGCTTCTCCGTCCATAA) (SEQ ID NO:30);

    (xxxii)   L-d(TTAGCTTCTCCGTCCATAA) (SEQ ID NO:23)

An especially preferred complementary sequence
comprises:

30   L-d(TTAGCTTCTCCGTCCATAA) (SEQ ID NO: 23)

The complementarity of the above cI L-enantiomeric
oligonucleotide sequences of structure V with respect to
the previously listed "I" L-enantiomeric oligonucleotide
sequences of structure IV depends on the relative

35   orientation of each, i.e., whether the sequences of I
and of cI are constructed from 5' to 3' or 3' to 5', or

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vice versa, respectively. Preferably, the Tm of a
hybridized complex formed between the respective I and
cI sequences is greater than 37°C.

In a preferred embodiment, the L-enantiomeric
oligonucleotide sequence cI can comprise double stranded
L-DNA or L-RNA. That is, the L-enantiomeric
oligonucleotide sequence may comprise complementary L-
DNA or L-RNA which forms a double helix molecule. The
complementary L-enantiomeric oligonucleotide sequence
incorporated into the non-radioactive targeting
immunoreagent composed of L-DNA or L-RNA then hybridizes
to one or the other of the strands of the double
stranded L-DNA or L-RNA comprising cI. In this way, the
complementary L-enantiomeric oligonucleotide sequence
incorporated into the non-radioactive targeting
immunoreagent interacts with the duplex L-DNA or L-RNA
of cI in such a way as to form triplex (triple helix) L-
DNA, triplex L-RNA, or a triplex L-DNA:L-RNA hybrid.

Q_{cI} in structure V is a spacing group; Q_{cI} can be
selected from Q_I as described for structure IV.
Preferably, Q_{cI} comprises an L-enantiomeric
oligonucleotide contiguous sequence of from 2 to about
30 L-enantiomeric nucleotides wherein the sequence of
which is not self-associating and wherein a contiguous
sequence of six or more L-enantiomeric nucleotide units
does not hybridize with any other contiguous sequence of
six or more contiguous L-enantiomeric nucleotide units
anywhere in structure V. Q_{cI} can also comprise an L-
enantiomeric oligonucleotide, preferably a sequence such
as (xxvii) to (xxxii) above which is complementary to
the "Sequence" in structure IV. Preferably, Q_{cI}
comprises one or two such sequences.

L_1, L_2, and L_3 in structure V are independently a
chemical bond, preferably a phosphate ester bond, or a
linking group which are defined as L_z and L_Q in the

above structure V. L₁, L₂, and L₃ can also
independently comprise components of QcI.

W₁, W₂, and W₃ in structure V are residues of
chelating groups. The chelating groups of this
invention can comprise the residue of one or more of a
wide variety of chelating agents that can have a
radionuclide associated therewith. As is well known, a
chelating agent is a compound containing donor atoms
that can combine by coordinate bonding with a metal atom
to form a cyclic structure called a chelation complex or
chelate. This class of compounds is described in the
Kirk-Othmer Encyclopedia of Chemical Technology, Vol. 5,
339-368.

The residues of suitable chelating agents can be
independently selected from polyphosphates, such as
sodium tripolyphosphate and hexametaphosphoric acid;
aminocarboxylic acids, such as
ethylenediaminetetraacetic acid, N-(2-
hydroxyethyl)ethylene-diaminetriacetic acid,
nitrilotriacetic acid, N,N-di(2-hydroxyethyl)glycine,
ethylenebis(hydroxyphenylglycine) and diethylenetriamine
pentacetic acid; 1,3-diketones, such as acetylacetone,
trifluoroacetylacetone, and thenoyltrifluoroacetone;
hydroxycarboxylic acids, such as tartaric acid, citric
acid, gluconic acid, and 5-sulfosalicylic acid;
polyamines, such as ethylenediamine, diethylenetriamine,
triethylenetetramine, and triaminotriethylamine;
aminoalcohols, such as triethanolamine and N-(2-
hydroxyethyl)ethylenediamine; aromatic heterocyclic
bases, such as 2,2'-dipyridyl, 2,2'-diimidazole,
dipicoline amine and 1,10-phenanthroline; phenols, such
as salicylaldehyde, disulfopyrocatechol, and
chromotropic acid; aminophenols, such as 8-
hydroxyquinoline and oximesulfonic acid; oximes, such as
dimethylglyoxime and salicylaldoxime; peptides
containing proximal chelating functionality such as

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polycysteine, polyhistidine, polyaspartic acid,
polyglutamic acid, or combinations of such amino acids;
Schiff bases, such as disalicylaldehyde 1,2-
propylenediimine; tetrapyrroles, such as

5   tetraphenylporphin and phthalocyanine; sulfur
compounds, such as toluenedithiol, meso-2,3-
dimercaptosuccinic acid, dimercaptopropanol,
thioglycolic acid, potassium ethyl xanthate, sodium
diethyldithiocarbamate, dithizone, diethyl

10   dithiophosphoric acid, and thiourea; synthetic
macrocylic compounds, such as dibenzo[18]crown-6,
(CH3)6-[14]-4,11-diene-N4, and (2.2.2-cryptate); and
phosphonic acids, such as nitrilotrimethylene-phosphonic
acid, ethylenediaminetetra(methylenephosphonic acid),

15   and hydroxyethylidenediphosphonic acid, or combinations
of two or more of the above agents.

Preferred residues of chelating agents contain
polycarboxylic acid groups and include: ethylenediamine-
N, N, N',N'-tetraacetic acid (EDTA); N,N,N',N",N"-

20   diethylene-triaminepentaacetic acid (DTPA); 1,4,7,10-
tetraazacyclododecane-N,N',N",N"'-tetraacetic acid
.(DOTA); 1,4,7,10-tetraazacyclododecane-N,N',N"-triacetic
acid (DO3A); 1-oxa-4,7,10-triazacyclododecane-N,N',N"-
triacetic acid (OTTA); trans(1,2)-

25   cyclohexanodiethylenetriamine pentaacetic acid (CDTPA);
Preferred residues of chelating agents contain
polycarboxylic acid groups and include the following:

B4A   P4A   DCDTPA

TMT   MacroTMT

PheMT   MacroPheMT
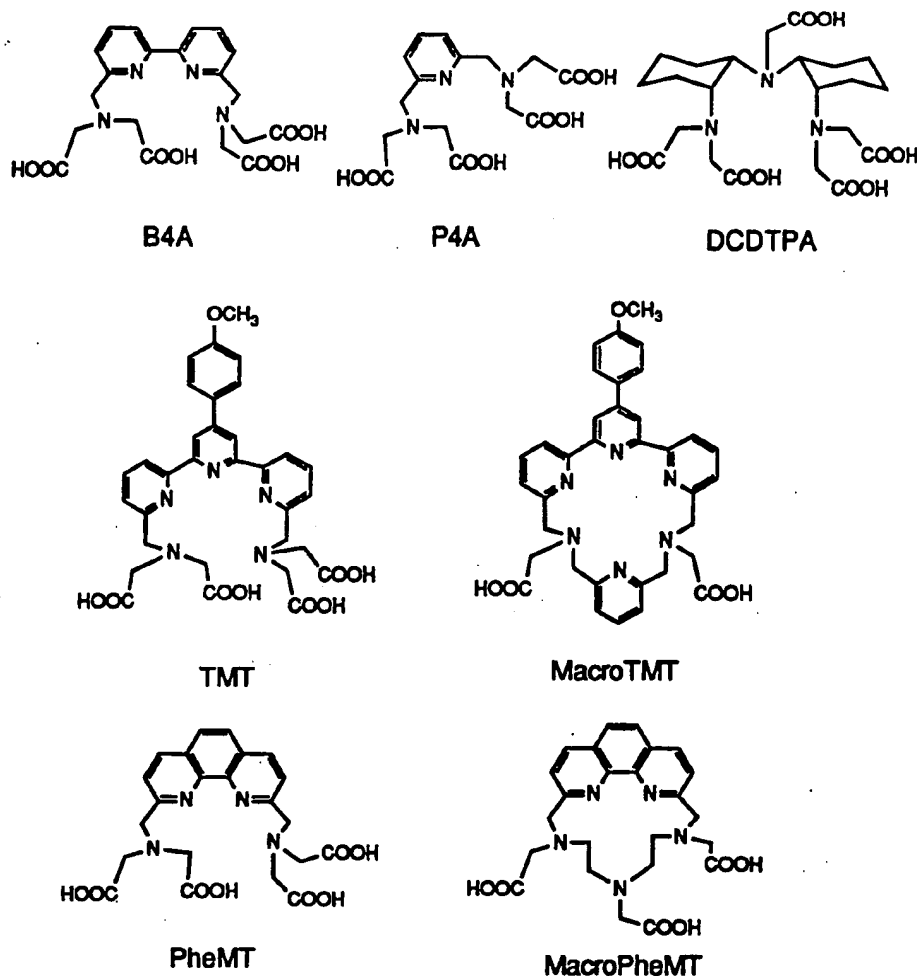
In one aspect, other suitable residues of chelating
agents comprise proteins modified for the chelation of
metals such as technetium and rhenium as described in
U.S. Patent No. 5,078,985, the disclosure of which is
hereby incorporated by reference.

In another aspect, suitable residues of chelating
agents are derived from N_3S and N_2S_2 containing
compounds, as for example, those disclosed in U.S.
Patent Nos. 4,444,690; 4,670,545; 4,673,562; 4,897,255;
4,965,392; 4,980,147; 4,988,496; 5,021,556 and
5,075,099.

Other suitable residues of chelating agents are
described in PCT/US91/08253, the disclosure of which is

hereby incorporated by reference. In structure V above,
if W_1, W_2, and W_3 comprise the residue of multiple
chelating agents, such agents can be linked together by
a linking group such as described above in structure IV.

5   A residue of each of the chelating agents W_1, W_2,
and W_3 in structure V is independently linked to the
complementary L-enantiomeric oligonucleotide moiety cI
or spacing group Q_{cI} through a chemical bond or a
linking group, i.e., L_1, L_2 and L_3 in structure V,
10   above. Preferred linking groups include nitrogen atoms
in groups such as amino, imido, nitrilo and imino
groups; alkylene, preferably containing from 1 to 18
carbon atoms such as methylene, ethylene, propylene,
butylene and hexylene, such alkylene optionally being
15   interrupted by 1 or more heteroatoms such as oxygen,
nitrogen and sulfur or heteroatom-containing groups;
carbonyl;
sulfonyl;
sulfinyl;
20   ether;
thioether;
ester, i.e., carbonyloxy and oxycarbonyl;
thioester, i.e., carbonylthio, thiocarbonyl,
thiocarbonyloxy, and oxythiocarboxy;
25   amide, i.e., iminocarbonyl and carbonylimino;
thioamide, i.e., iminothiocarbonyl and
thiocarbonylimino;
thio;
dithio;
30   phosphate; phosphonate;
urelene;
thiourelene;
urethane, i.e., iminocarbonyloxy, and oxycarbonylimino;
thiourethane, i.e., iminothiocarbonyloxy and
35   oxythiocarbonylimino;
an amino acid linkage, i.e., a

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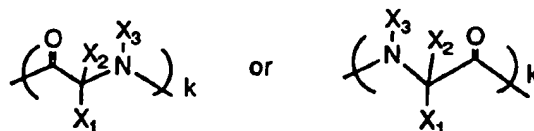
group wherein k=1 and X_1, X_2, X_3 independently are H, alkyl, containing from 1 to 18, preferably 1 to 6 carbon atoms, such as methyl, ethyl and propyl, such alkyl optionally being interrupted by 1 or more heteroatoms such as oxygen, nitrogen and sulfur, substituted or unsubstituted aryl, containing from 6 to 18, preferably 6 to 10 carbon atoms such as phenyl, hydroxyiodophenyl, hydroxyphenyl, fluorophenyl and naphthyl, aralkyl, preferably containing from 7 to 12 carbon atoms, such as benzyl, heterocyclyl, preferably containing from 5 to 7 nuclear carbon and one or more heteroatoms such as S, N, P or O, examples of preferred heterocyclyl groups being pyridyl, quinolyl, imidazolyl and thienyl; heterocyclylalkyl, the heterocyclyl and alkyl portions of which preferably are described above; or a peptide linkage, i.e., a
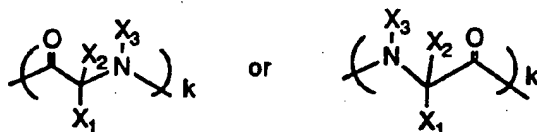
group wherein k>1 and each of X_1, X_2, and X_3 is independently represented by a group as described for X_1, X_2, and X_3 above. Two or more linking groups can be used, such as, for example, alkyleneimino and iminoalkylene. It is contemplated that other linking groups may be suitable for use herein, such as linking groups commonly used in protein heterobifunctional and homobifunctional conjugation and crosslinking chemistry as described for L_Z or L_Q in structure IV.

Especially preferred linking groups include
unsubstituted or substituted phosphate ester groups
containing amino groups which when linked to the residue
of a chelating agent via an isothiocyanate group on the
5   chelating agent form a thiourea group.

The linking groups can contain various substituents
which do not interfere with the coupling reaction
between chelate W_1, W_2, or W_3 and L-enantiomeric
oligonucleotide cI or the spacing group. The linking
10   groups can also contain substituents which can otherwise
interfere with such reaction, but which during the
coupling reaction, are prevented from so doing with
suitable protecting groups commonly known in the art and
which substituents are regenerated after the coupling
15   reaction by suitable deprotection. The linking groups
can also contain substituents that are introduced after
the coupling reaction. For example, the linking group
can be substituted with a group such as a halogen, such
as F, Cl, Br or I; an ester group; an amide group;
20   alkyl, preferably containing from 1 to about 18, more
preferably, 1 to 4 carbon atoms such as methyl, ethyl,
propyl, i-propyl, butyl, and the like; substituted or
unsubstituted aryl, preferably containing from 6 to
about 20, more preferably 6 to 10 carbon atoms such as
25   phenyl, naphthyl, hydroxyphenyl, iodophenyl,
hydroxyiodophenyl, fluorophenyl and methoxyphenyl;
substituted or unsubstituted aralkyl, preferably
containing from 7 to about 12 carbon atoms, such as
benzyl and phenylethyl; alkoxy, the alkyl portion of
30   which preferably contains from 1 to about 18 carbon
atoms as described for alkyl above; alkoxyaralkyl, such
as ethoxybenzyl; substituted or unsubstituted
heterocyclyl, preferably containing from 5 to 7 nuclear
carbon and heteroatoms such as S, N, P or O, examples of
35   preferred heterocyclyl groups being pyridyl, quinolyl,
imidazolyl and thienyl; a carboxyl group; a carboxyalkyl

55

group, the alkyl portion of which preferably contains
from 1 to 8 carbon atoms; or the residue of a chelating
group.

In structure V, M_1, M_2 and M_3 each comprise
elements with oxidation states equal to or greater than
+1, and at least one of which is a radionuclide.
Preferably each of M_1, M_2 and M_3 comprise a metal
isotope, preferably a radioactive metal isotope,
sometimes herein referred to as a metal radioisotope,
which radioisotope is useful in a therapeutic or in a
diagnostic imaging application. Preferred metal
radioisotopes are selected from, for example, Sc, Fe,
Pb, Ga, Y, Bi, Mn, Cu, Cr, Zn, Ge, Mo, Tc, Ru, In, Sn,
Re, Sr, Sm, Lu, Eu, Ru, Dy, Sb, W, Re, Po, Ta and Tl.
Useful emissions from such radioisotopes include
spontaneous alpha emissions, beta emissions, gamma
emissions, X-ray emissions, positron emissions, and such
emissions as are induced by the processes of electron
capture and internal conversion. Said emissions can be
purely of one kind such as pure alpha, pure beta, pure
gamma and the like, or of combinations of nuclear
emissions such as beta and gamma emissions and the like.

Radioisotopes with emissions comprising, for
example, alpha radiation or beta radiation are useful in
therapeutic applications, especially in the therapy of
cancer. Useful isotopes in therapeutic applications
include, for example, alpha radiation emitting isotopes
such as, for example, ^{207}Pb, ^{211}Pb, ^{208}Pb, ^{212}Pb, ^{212}Bi,
^{207}Ti, and ^{223}Ra; beta radiation emitting isotopes such
as, for example ^{47}Sc, ^{66}Ga, ^{67}Cu, ^{77}As, ^{90}Y, ^{105}Rh,
^{109}Pd, ^{111}Ag, ^{121}Sn, ^{127}Te, ^{143}Pr, ^{149}Pm, ^{153}Sm, ^{161}Tb,
^{166}Ho, ^{169}Er, ^{177}Lu, ^{188}Re, ^{186}Re, ^{191}Os and ^{199}Au; and
isotopes which emit radiation as a result of the
processes of electron capture and internal conversion
such as, for example, ^{97}Ru, ^{177m}Sn, ^{199}Sb, ^{128}Ba and
^{197}Hg. Radioisotopes especially preferred in

therapeutic applications include ^{212}Pb, ^{212}Bi, ^{90}Y, ^{177}Lu, ^{186}Re, and ^{188}Re. Currently the most preferred radioisotope is ^{90}Y.

Radioisotopes with emissions comprising, for example, gamma radiation or positron radiation are useful in diagnostic imaging applications, especially in diagnostic imaging of cancer. Useful isotopes in diagnostic imaging applications include, for example, gamma radiation emitting isotopes such as ^{47}Sc, ^{51}Cr, ^{67}Cu, ^{67}Ga, ^{97}Ru, ^{99m}Tc, ^{111}In, ^{117m}Sn, ^{141}Ce, ^{167}Tm, ^{199}Au, ^{87}Y and ^{203}Pb; and positron radiation emitting isotopes such as ^{44}Sc, ^{48}V, ^{64}Cu, ^{66}Ga, ^{69}Ge, ^{72}As, ^{86}Y and ^{89}Zr. Radioisotopes especially preferred in diagnostic imaging applications include ^{64}Cu, ^{99m}Tc, ^{111}In and ^{87}Y. Currently, the most preferred are ^{99m}Tc and ^{111}In.

In another aspect, other suitable radionuclides can be incorporated, for example, by covalent bonding into Q_{cI} and include radioactive isotopes of halogens such as radioactive isotopes of iodine, for example, ^{123}I, ^{124}I, ^{125}I and ^{131}I as well as radioactive isotopes of astatine such as ^{211}At.

Methods of generating an image useful in the diagnostic imaging of, for example, cancer in a mammal comprise detecting emissions imagewise from radioisotopes as employed in the compositions and methods of this invention. Said image generating methods comprise the use of, for example, a collimated camera detector such as a gamma camera commonly employed in radioimmunoscintigraphy (RIS), and the use of linked X-ray detectors commonly employed in positron emission tomography (PET) and in single photon emission tomography (SPET).

In structure V, x, y, and z are independently zero or 1 provided that at least one of x, y, or z is one; and

w and b are zero or an integer from 1 to about 4.

Preferred compositions can be prepared as outlined
in the schemes that follow.

5

Scheme 6

Derivatization of antibody amine groups with
heterobifunctional linking reagents SMCC, 2-IT, or SATA.
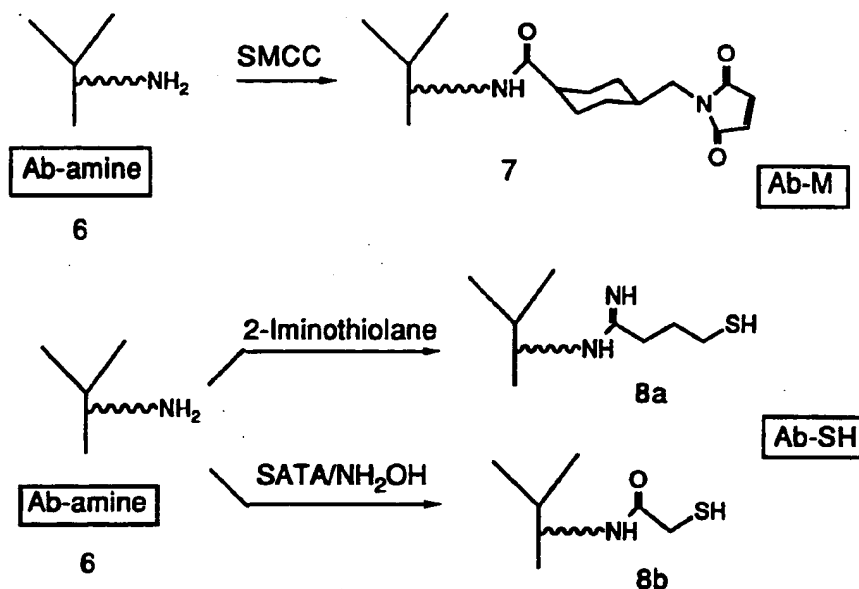
10

In scheme 6, the protein (antibody such as ING-1,
antibody fragment, enzyme, receptor) is chemically
modified for later covalent coupling to a thiolated L-
enantiomeric oligonucleotide (to Ab-M) or to a
15   maleimido-group-containing L-enantiomeric
oligonucleotide (to Ab-SH). Chemical modification is
effected using a bifunctional cross linking agent,
preferably a heterobifunctional cross linking agent
having both a group capable of reacting with protein
20   functional groups (e.g. amine in Ab-amine) and also
having a further group capable of reacting with thiol
groups. The latter is selected from haloacetyl, halo-

acetamidyl, maleimido, and activated disulfide functions.

Maleimido and thioalkyl groups are introduced to an antibody by utilizing the heterobifunctional linkers,

5 sulfosuccinimido-4-(M-maleimidomethyl)-cyclohexane-1-carboxylate (Sulfo-SMCC), 2-iminothiolane (2-IT), or succinimidyl-S-acetylthioacetate(SATA). The reaction of a sample containing antibody with a linking agent is for a time sufficient to introduce an average of about 0.5-3

10 linking agent molecules per antibody molecule in the sample. The derivatized antibody is purified using a gel filtration column, and more preferably a Sephadex G-25 column.

Non-limiting examples of preferred protein

15 conjugates are listed below:

ING-1-NH-CO-cyclohexane-CH_2-Maleimide;

ING-1-NH-C(=NH)-$(CH_2)_3$-SH; and

ING-1-NH-CO-CH_2-SH.

ING-1 is a preferred protein containing amine groups

20 such as lysine amines as represented by Ab-amine.

Scheme 7

Derivatization of L-enantiomeric oligonucleotide "L-d(I-Q_I-I)" via amine groups with heterobifunctional linking reagents SATA and SMCC.

5

L-d(Oligo)-3'-NH_2

wherein [P] is a phosphate ester group

SATA
NH_2OH

L-d(Oligo)-3'-SH

SMCC

L-d(Oligo)-3'-M
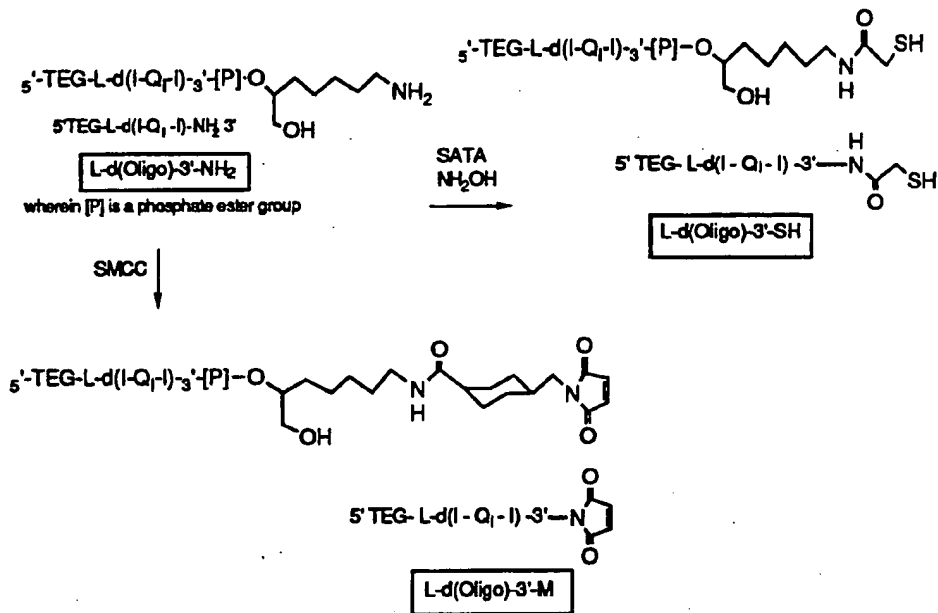
Scheme 8

Derivatization of L-enantiomeric oligonucleotide "L-d(I-Q$_I$-I)" with amine groups and with sulfhydryl groups for use with heterobifunctional linking reagents.

5

[for 5' attachment]

[for 3' end blocked]

[Tr = trityl]   Ag$^+$

[DMT = dimethoxy trityl]

HS—...—O[P]-5'-L-d(I-Q$_I$-I)-3'-[P]-O—...—NH$_2$

L-d(Oligo)-5'-SH
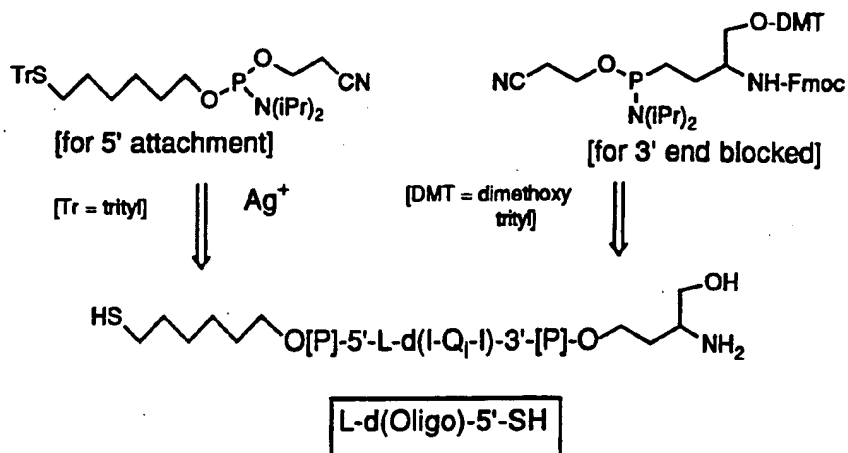
5'HS-L-d(I-Q$_I$-I)-NH$_2$ 3'

Scheme 9

Derivatization of L-enantiomeric oligonucleotide "5'-TrS-L-d(I-Q$_I$-I)-3'-NH2" with biotin for use with heterobifunctional linking reagents.

10

TrS-5'-L-d(Oligo)-3'-NH$_2$
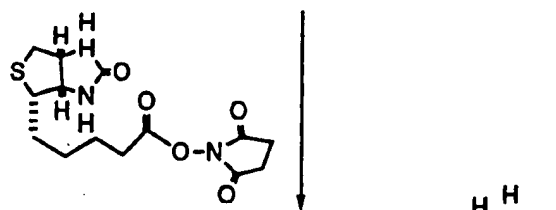
TrS-5'-L-d(Oligo)-3'-NH

TrS-5'-L-d(I-Q$_I$-I)-3'-Biotin

TrS-5'-L-d(Oligo)-3'-Biotin

Ag$^+$
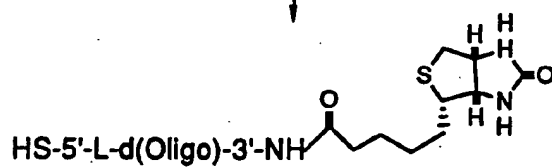
HS-5'-L-d(Oligo)-3'-NH

HS-5'-L-d(I-Q$_I$-I)-3'-Biotin

HS-5'-L-d(Oligo)-3'-Biotin

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<u>Scheme 10</u>

Preparation of "5'-HS-L-d(I-Q$_I$-I)-3'-biotin" with
biotinylated phosphoramidite reagent for use with
heterobifunctional linking reagents.

[for 3' end blocked]

Solid Phase Synthesis of
L-d(I-Q$_r$-I)-3'-Biotin

HO-5'-L-d(I-Q$_r$-I)-3'-Biotin

[for 5' attachment]

Ag$^+$

HS-5'-L-d(I-Q$_l$-I)-3'-Biotin
HS-5'-L-d(Oligo)-3'-Biotin
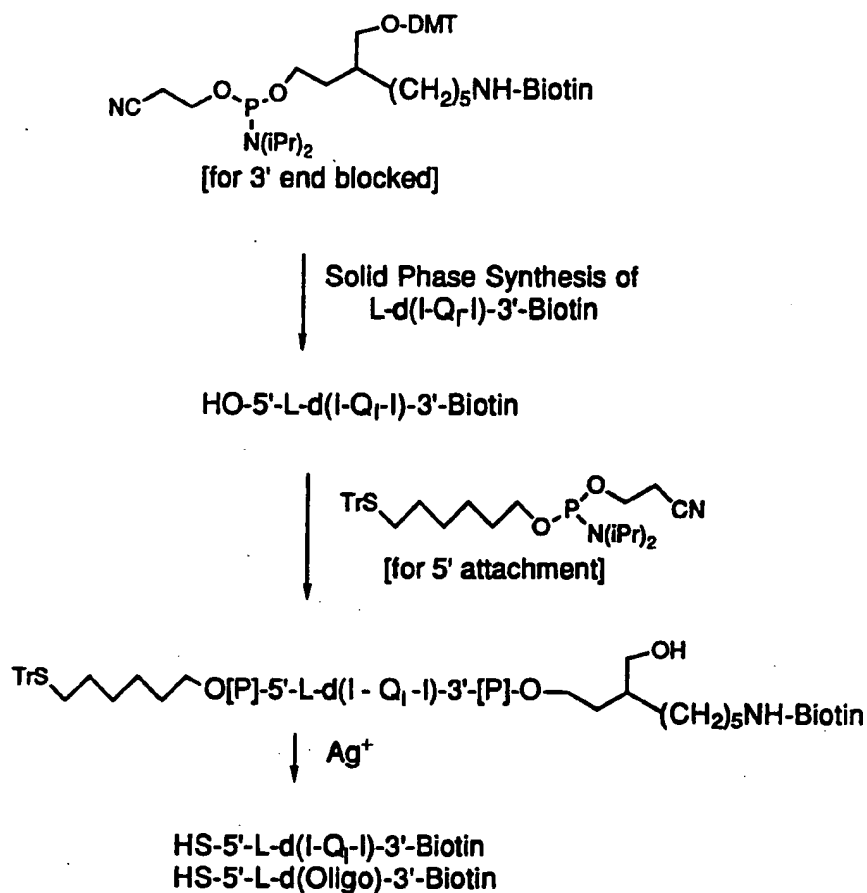
L-enantiomeric oligonucleotides and modified L-
enantiomeric oligonucleotides are synthesized according
to standard methods such as solid phase synthesis that
are well known in the art for the synthesis of D-
enantiomeric oligonucleotides. Derivatizations of L-
enantiomeric oligonucleotides L-d(I-Q$_I$-I) are achieved
using the reaction of 5'-TEG-L-enantiomeric oligomer-
NH$_2$-3' with SATA or SMCC to afford L-enantiomeric
oligomer-3'-SH or L-enantiomeric oligomer-3'-M (see
Scheme 7).

63

The following L-enantiomeric oligonucleotides are preferred:

(a) L-d(TTATGGACGGAGAAGCTAA) (SEQ ID NO: 8), and

(b) {5'-Teg-L-d(TCTTATGGACGGAGAAGCTAA-[ACTCTC]-
            TTATGGACGGAGAAGCTAATCT)-3'-amine-L-T},
(SEQ ID No:31)

wherein the square bracketed portion of SEQ ID No:31 denotes the preferred spacer, -L-d[ACTCTC]-, which separates two sequences each containing the preferred sequence -L-d(TTATGGACGGAGAAGCTAA)- (SEQ ID No: 8).

These L-enantiomeric oligomers are also derivatized to afford a bifunctionalized L-enantiomeric oligomer: 5'-HS-L-d(I-Q$_I$-I)-NH$_2$-3' via introduction of bifunctional reagents at the 5'- and 3'-positions, as shown in Scheme 8. The introduction of biotin at the 3'-position is also achieved by the reaction of 5'-HS-L-d(I-Q$_I$-I)-NH$_2$-3' with biotin-imidocarboxylate as shown in Scheme 9.

There are useful spacers with a protected amine, thiol, or carboxyl group on one end and a phosphoramidite at the other (see Scheme 10).

The applications of these spacers are shown in reactions below.

Scheme 11

Assembly of Ab-M + L-d(Oligo)-5'-SH

Ab-M

5' HS-L-d(I -Q$_I$ -I)-NH$_2$ 3'

Ab-M-5'-S-L-d(Oligo)-3'-NH$_2$
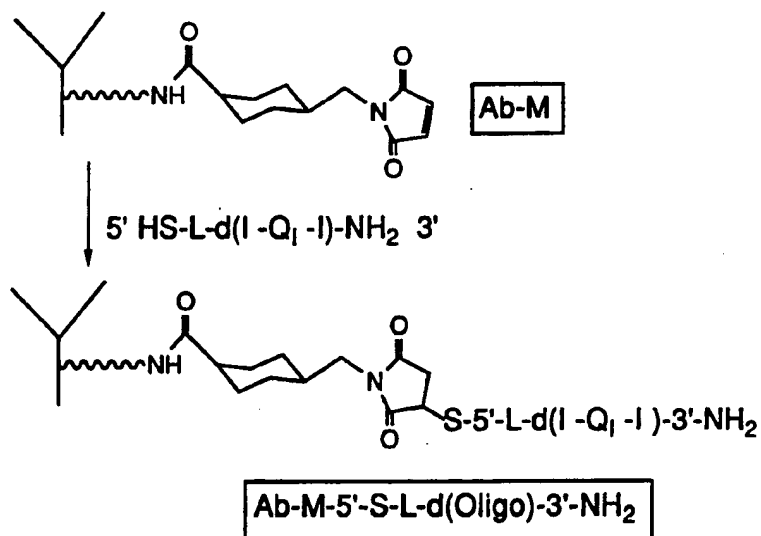
Scheme 12

Assembly of Ab-SH + L-d(Oligo)-3'-M

5'TEG-L-d(I -Q- I) -3'-N

Ab-S-M-3'-L-d(Oligo)
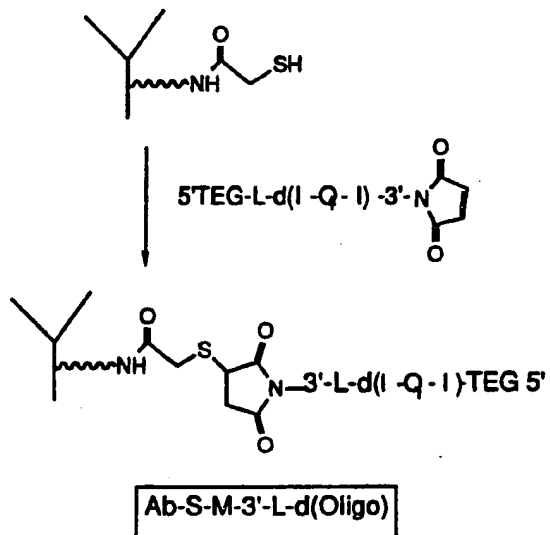
The modified maleimido antibody (Ab-M, Scheme 6)
and the thiolated L-enantiomeric oligonucleotide (5'-HS-
L-d(I-Q$_I$-I)-NH2-3') can be assembled to yield the
modified antibody-L-enantiomeric oligonucleotide

conjugate (Ab-M-5'-S-succinimido-L-d(I-Q$_I$-I)-NH$_2$-3') as shown in Scheme 11. Similarly, Ab-S-M-3'-L-d(I-Q$_I$-I)-TEG-5' is prepared from Ab-SH and 5'-TEG-L-d(I-Q$_I$-I)-maleimide-3' as shown in Scheme 13.

5

Scheme 13

Complementary L-Oligonucleotide "5'-TMT-L-d(cl)-TMT-3'"

To form 5' amine

2 TMT-NCS

5'H$_2$N-L-d(cl)-NH$_2$ 3' ⟶ 5'TMT-L-d(cl)-TMT 3'

To form 3' amine
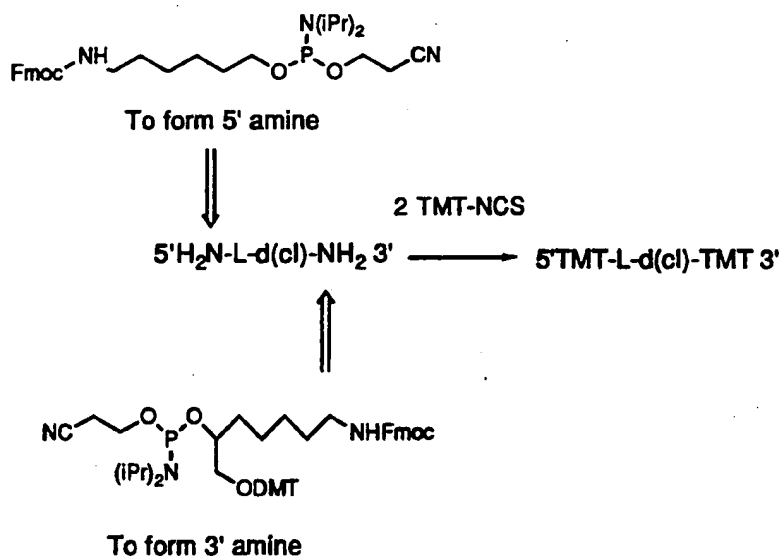
10   In these schemes, L-d(oligo) represents an L-enantiomeric oligodeoxyribonucleotide such as an L-deoxyribonucleotide, L-d(I); d(cI) represents the complementary L-enantiomeric oligodeoxyribonucleotide sequence; L-d(I-Q$_I$-I) is an L-enantiomeric

15   oligodeoxyribonucleotide; and TMT represents a member of a class of terpyridine chelates, preferably as described above. A preferred TMT is 4'-(3-isothiocyanato-4-methoxyphenyl)-6,6"-bis[N,N-di(carboxymethyl)-aminomethyl]-2,2':6',2"-terpyridine, TMT-NCS.

20   The reaction of 5'-H$_2$N-L-d(cI)-NH$_2$-3' with 2 moles of TMT-NCS affords the desired 5'-TMT-L-d(cI)-TMT-3' as shown in Scheme 13.

There are other useful linking agents with a
protected amine, thiol, or carboxyl group on one end and
a phosphoramidite at the other. The applications of
these agents are shown in the Examples.

5   In a preferred embodiment, an effective dose of a
radioactive targeting reagent as described above in a
pharmaceutically acceptable medium is prepared by
exposing a composition comprising a complementary L-
enantiomeric oligonucleotide sequence containing one or

10   more chelating groups such as the
oligodeoxyribonucleotide sequence as described above to
a composition containing a radioactive metal isotope
such that the molar amount of the radionuclide metal
isotope is less than the molar amount of the chelating

15   groups. The exposure lasts an effective time during
which uptake of of the radionuclide metal isotope into
the chelating agents occurs.

In a preferred embodiment, an effective dose of a
non-radioactive targeting immunoreagent as described

20   above in a pharmaceutically acceptable medium is
administered to a patient and the non-radioactive
targeting immunoreagent is allowed to accumulate at the
target site such as at a tumor site in the patient.
Subsequently, an effective dose of a radioactive

25   targeting reagent as described above in a
pharmaceutically acceptable medium is administered to
the patient, and the radioactive targeting reagent is
allowed to accumulate at the target site, said target
site being the non-radioactive targeting immunoreagent

30   which has accumulated at the tumor site in the patient.

The present invention also comprises one or more of
the immunoreagents of this invention formulated into
compositions together with one or more non-toxic
physiologically acceptable carriers, adjuvants or

35   vehicles which are collectively referred to herein as
carriers, for parenteral injection for oral

administration in solid or liquid form, for rectal or topical administration, or the like.

The compositions can be administered to humans and animals either orally, rectally, parenterally

5   (intravenously, intramuscularly or subcutaneously), intracisternally, intravaginally, intraperitoneally, intravesically, intra-articularly, locally (in the form of powders, ointments or drops), or as a buccal or nasal spray.

10   Compositions suitable for parenteral injection may comprise physiologically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples

15   of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (propylene glycol, polyethylene glycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such

20   as ethyl oleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants.

These compositions may also contain adjuvants such

25   as preserving, wetting, emulsifying, and dispensing agents. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to

30   include isotonic agents, for example sugars, sodium chloride and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

35   Solid dosage forms for oral administration include capsules, tablets, pills, powders and granules. In such

solid dosage forms, the active compound is admixed with
at least one inert customary excipient (or carrier) such
as sodium citrate or dicalcium phosphate or (a) fillers
or extenders, as for example, starches, lactose,
sucrose, glucose, mannitol and silicic acid, (b)
binders, as for example, carboxymethylcellulose,
alignates, gelatin, polyvinylpyrrolidone, sucrose and
acacia, (c) humectants, as for example, glycerol, (d)
disintegrating agents, as for example, agar-agar,
calcium carbonate, potato or tapioca starch, alginic
acid, certain complex silicates and sodium carbonate,
(e) solution retarders, as for example paraffin, (f)
absorption accelerators, as for example, quaternary
ammonium compounds, (g) wetting agents, as for example,
cetyl alcohol and glycerol monostearate, (h) adsorbents,
as for example, kaolin and bentonite, and (i)
lubricants, as for example, talc, calcium stearate,
magnesium stearate, solid polyethylene glycols, sodium
lauryl sulfate or mixtures thereof. In the case of
capsules, tablets and pills, the dosage forms may also
comprise buffering agents.

   Solid compositions of a similar type may also be
employed as fillers in soft and hard-filled gelatin
capsules using such excipients as lactose or milk sugar
as well as high molecular weight polyethylene glycols,
and the like.

   Solid dosage forms such as tablets, dragees,
capsules, pills and granules can be prepared with
coatings and shells, such as enteric coatings and others
well known in the art. They may contain opacifying
agents, and can also be of such composition that they
release the active compound or compounds in a certain
part of the intestinal tract in a delayed manner.
Examples of embedding compositions which can be used are
polymeric substances and waxes.

The active compounds can also be in micro-encapsulated form, if appropriate, with one or more of the above-mentioned excipients.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art, such as water or other solvents, solubilizing agents and emulsifiers, as for example, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils, in particular, cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil and sesame oil, glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan or mixtures of these substances, and the like.

Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring and perfuming agents. Suspensions, in addition to the active compounds, may contain suspending agents, as for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, or mixtures of these substances, and the like.

Compositions for rectal administrations are preferably suppositories which can be prepared by mixing the compounds of the present invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax, which are solid at ordinary temperatures but liquid at body temperature and, therefore, melt in the rectum or vaginal cavity and release the active component.

Dosage forms for topical administration of a
compound of this invention include ointments, powders,
sprays and inhalants. The active component is admixed
under sterile conditions with a physiologically

5   acceptable carrier and any preservatives, buffers or
propellants as may be required. Ophthalmic
formulations, eye ointments, powders and solutions are
also contemplated as being within the scope of this
invention.

10   Actual dosage levels of active ingredient in the
compositions of the present invention may be varied so
as to obtain an amount of active ingredient that is
effective to obtain a desired therapeutic response for a
particular composition and method of administration.

15   The selected dosage level therefore depends upon the
desired therapeutic effect, on the route of
administration, on the desired duration of treatment and
other factors.

The total daily therapeutic dose of the compounds

20   of this invention administered to a host in a single or
divided dose may be in amounts, for example, of from
about 100 picomol to about 5 micromols per kilogram of
body weight. Dosage unit compositions may contain such
amounts of such submultiples thereof as may be used to

25   make up the daily dose. It will be understood, however,
that the specific dose level for any particular patient
will depend upon a variety of factors including the body
weight, general health, sex, diet, time and route of
administration, rates of absorption and excretion,

30   combination with other drugs and the severity of the
particular disease being treated.

In another embodiment, the present invention is
directed to a method of diagnosis comprising the
administration of a diagnostic imaging effective amount

35   of the compositions of the present invention to a mammal
in need of such diagnosis. A method for diagnostic

imaging for use in medical procedures in accordance with
this invention comprises administering to the body of a
test subject in need of a diagnostic image an effective
diagnostic image producing amount of the above-described

5   compositions.   In this method, an effective diagnostic
image producing amount of a non-radioactive targeting
immunoreagent as described above in a pharmaceutically
acceptable medium is administered to a patient and said
non-radioactive targeting immunoreagent is allowed to

10   accumulate at the target site such as at a tumor site in
said patient. Subsequently, a diagnostic imaging
effective dose of a radioactive targeting reagent as
described above in a pharmaceutically acceptable medium
is administered to said patient, and said radioactive

15   targeting reagent is allowed to accumulate at the target
site, said target site being the said non-radioactive
targeting immunoreagent accumulated at said tumor site
in said patient. The image pattern can then be
visualized.

20   The total diagnostic imaging effective dose of the
compounds of this invention administered to a host in a
single or divided dose may be in amounts, for example,
of from about 1 picomol to about 0.5 micromols per
kilogram of body weight. Dosage unit compositions may

25   contain such amounts of such submultiples thereof as may
be used to make up the effective diagnosting imaging
dose. It will be understood, however, that the specific
dose level for any particular patient will depend upon a
variety of factors including the body weight, general

30   health, sex, diet, time and route of administration,
rates of absorption and excretion, combination with
other drugs and the severity of the particular disease
being treated.

            In addition to human patients, the test subjects
35   can include mammalian species such as rabbits, dogs,

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cats, monkeys, sheep, pigs, horses, bovine animals and
the like.

      After administration of the compositions of the
present invention, the subject mammal is maintained for
5   an effective time which is a time period sufficient for
the administered compositions to be distributed
throughout the subject and to enter the targeted tissues
of the mammal. A sufficient time period for the non-
radioactive targeting immunoreagent is generally from
10   about 1 hour to about 2 weeks or more and, preferably
from about 2 hours to about 1 week. A sufficient time
period for the radioactive targeting reagent such as the
preferred ^{90}Y is generally measured in terms of half-
life of the radionuclide and as such is in the range of
15   from about 1 to about 10 half-lives or more and,
preferably from about 2 hours to about 6 half-lives.

      The following examples further illustrate the
invention and are not to be construed as limiting of the
specification and claims in any way. Specific
20   embodiments of the invention are illustrated in the
following examples.

<div align="center">EXAMPLES</div>

Example 1
25   L-Enantiomeric Oligonucleotide Design

      A targeting immunoreagent of this invention as
described in Structure IV comprising an antibody Z,
linking groups L_Z and L_Q, an L-enantiomeric
oligonucleotide sequence I, a spacing group Q_I, a second
30   sequence I_i, and an end capping group E is designed as
follows utilizing anticipated complementary binding
properties between complementary pairs of mirror image
D-enantiomeric oligonucleotides as a model for analogous
physical properties imputed to the desired L-
35   enantiomeric oligonucleotide.

<div align="center">73</div>

A sample mirror image, naturally occurring D-enantiomeric oligodeoxyribonucleotide with the following sequence (herein referred to as SEQ ID NO: 32) is analyzed for conformity to the criteria described previously for Structure IV wherein groups L_Z, I, Q_I, I_i, and L_Q and E are as represented below as D-enantiomers:

L_Z	I_Z	Q_I	I_i	L_Q	E

5'D-

[TC|TTATGGACGGATCCGCTAA|TC|TTATGGACGGATCCGCTAA|TCT]3'

(mirror image D-enantiomer SEQ ID NO: 32)

Analysis of this D-enantiomeric oligonucleotide sequence using "oligo" computer software (National Biosciences) designed for D-enantiomers reveals it to contain 5 regions of self-complementarity. All such regions are hairpin loops with differing degrees of overlap. The sum of the negative free energy changes (dG's) for these self-complementary regions is -28.3 kcal/mole which predicts a melting temperature of 86°C in 1.0 molar salt for this D-enantiomeric oligonucleotide. The most stable region of self-complementarity (dG = -15 kcal/mol) contains 8 base pairs most of which correspond to a BAM-1 restriction site palindrome. In the presence of these regions of self-complementarity a D-enantiomeric oligonucleotide sequence complementary to this sequence cannot hybridize successfully to its full length. By inference, a mirror image L-enantiomeric oligonucleotide of the same sequence and an L-enantiomeric oligonucleotide sequence complementary to the L-enantiomeric oligonucleotide sequence can also not hybridize successfully to its full length in the presence of mirror image regions of self-complementarity. Modifications to the D-enantiomeric oligonucleotide sequence (as well as respective modifications to the mirror image L-enantiomeric oligonucleotide) are then made in accordance with the

criteria outlined for structure IV to replace selected
bases in regions of self-complementarity such that
excessive base pairing is removed in iterative analysis.
Additional D-enantiomeric and additional L-enantiomeric
5   nucleotides are also inserted into the respective spacer
sequences to ensure that, in helical conformations, the
respective terminal groups of two respective
complementary sequences, D-cI and L-cI, when hybridized
to the two D-enantiomer sequences I and II and to the
10   two L-enantiomer sequences, respectively are orthogonal
to each other.

In this manner, a new D-enantiomeric
oligonucleotide (SEQ ID NO: 33) with the following
sequence is designed. The mirror image L-enantiomer
15   (SEQ ID NO: 34) is synthesized and the L-enantiomer is
conjugated to an immunoreactive molecule (i.e., to an
antibody such as ING-1):

                        D-Enantiomer Oligonucleotide

20   Lz   I   QI   Ii
LQ   E
 5'X-
D[TC|TTATGGACGGAGAAGCTAA|ACTCTC|TTATGGACGGAGAAGCTAA|TCTY
T]-3'

25   (SEQ ID NO: 33)

                        L-Enantiomer Oligonucleotide
        L-d(Lz   I   QI   Ii
LQ   E)
30   5'X-L-
d(TC|TTATGGACGGAGAAGCTAA|ACTCTC|TTATGGACGGAGAAGCTAA|TCTYT)-
3'

                                          (SEQ ID NO: 34)

35   This L-enantiomeric oligonucleotide (SEQ ID NO: 34)
comprises two copies of the same L-enantiomeric

nucleotide sequence (i.e., I = I_i = L-
d(TTATGGACGGAGAAGCTAA); SEQ ID NO: 8) linked through 6
L-enantiomeric nucleotide spacer [Q_I = L-d(ACTCTC)].
The groups L_Z and L_{QE} at the 5' or the 3' end comprise

5   groups X and Y. X and Y can each independently be an
amine-containing group (such as, for example, those
available from Clontech Industries, each of which amine-
containing groups being sometimes hereinafter
cryptically referred to as "NH$_2$" or as "amine") or one

10   of X and Y is selected from an amine-containing group
and the other is selected from one or more TEG groups as
described above. Additionally, one of X and Y can also
be a terminal hydroxyl group (in which case the 3'-YT in
the above sequence becomes a deoxyribosyl-OH group on

15   removal from the solid phase support, and more
preferably X is a 5'OH group available by removal of a
DMT group with acid at the end of the synthetic sequence
before deprotecting the amine groups with ammonium
hydroxide), or one of X and Y can also be a terminal

20   phosphate ester group (in which case the 3'-YT or the
5'-X becomes a -OPO$_3$H$_2$ group or an ionized salt such as
a sodium salt thereof), as desired. Such phosphate
terminal groups can be made by treatment of the 5'-OH
terminal group with 2-cyanoethyl N,N-diisopropyl

25   chlorophosphoramidite at the end of the synthesis.
Subsequent reaction with ammonium hydroxide and
oxidation provides the terminal phosphate group at X. A
3'-phosphate group can be introduced after synthesis of
the oligomer and its removal from the solid support by

30   treatment of the 3-hydroxyl group with phosphoric acid
anhydide followed by hydrolysis in water.
        Thus, in one embodiment, this L-enantiomeric
oligonucleotide, cryptically referred to as 5'-Teg-L-
d(I-QI-I)-3'-amine, is:

35   5'Teg-L-
d(TCTTATGGACGGAGAAGCTAAACTCTCTTATGGACGGAGAAGCTAATCT)-3'-

                              76

NH2-T, i.e., (SEQ ID NO: 31), or 5'-Teg-L-d(I-Q_I-I)-
3'amine.

In another embodiment, this L-enantiomeric
oligonucleotide, cryptically referred to as 5'-amine-L-

5   d(I-Q_I-I)-3'Teg, is:
5'H2N-L-
d(TCTTATGGACGGAGAAGCTAAACTCTCTTATGGACGGAGAAGCTAATCT-3'-
Teg-T) or 5'-amine-L-d(I-Q_I-I)-3'-Teg or (SEQ ID NO: 35)

In another embodiment, this L-enantiomeric

10   oligonucleotide, cryptically referred to as 5'-amine-L-
d(I-Q_I-I)-3'OH, is:
5'H2N-L-
d(TCTTATGGACGGAGAAGCTAAACTCTCTTATGGACGGAGAAGCTAATCT-3'-
OH) or 5'-amine-L-d(I-Q_I-I)-3'-OH of (SEQ ID NO: 36)

15   In another embodiment, this L-enantiomeric
oligonucleotide, cryptically referred to as 5'-amine-L-
d(I-Q_I-I)-3'OPO3H2, is:
5'H2N-L-
d(TCTTATGGACGGAGAAGCTAAACTCTCTTATGGACGGAGAAGCTAATCT-3'-

20   OPO3H2) or 5'-amine-L-d(I-Q_I-I)-3'-OPO3H2 or (SEQ ID NO:
37).

Example 2
(2a) Synthesis of L-d(I-Q_I-I); 5'-Teg-L-d(I-Q_I-I)-3'-NH2

25   (SEQ ID NO: 31)
This L-enantiomeric oligonucleotide is prepared on
an Applied Biosystems oligonucleotide synthesizer
originally designed by the manufacturer to be used for
the synthesis of naturally occurring D-enantiomeric

30   oligonucleotides. The method applicable to the trityl-
on protocol for the synthesis of naturally occurring D-
enantiomeric oligonucleotides is used as directed by the
equipment manufacturer but is modified to instead use L-
2-deoxynucleotide phosphoramidite reagent precursors 5'-

35   dimethoxytrityl L-cytidine-3'-O-phosphoramidite, 5'-
dimethoxytrityl L-adenosine-3'-O-phosphoramidite, 5'-

dimethoxytrityl L-guanosine-3'-O-phosphoramidite, and
5'-dimethoxytrityl L-thymidine-3'-O-phosphoramidite
prepared as described above rather than the D-
enantiomers. Clonetech's Uni-link Amino Modifier is

5   used as the precursor to the 3'-amine group. The TEG
group, a tetra(ethylene glycol) phosphate diester linked
in this invention by a phosphate ester bond to 5'-
dimethoxytrityl L-thymidine-3'-O-phosphoramidite [which
is the L-enantiomer (mirror image) of the reagent

10   disclosed in WO/92/02534 which refers to a
tetra(ethylene glycol) phosphate diester linked by a
phosphate ester bond to 5'-dimethoxytrityl D-thymidine-
3'-O-phosphoramidite] is used at the 5'-end of the
strand.   Following synthesis of the entire L-

15   enantiomeric oligonucleotide, the base protecting groups
and solid support are removed with ammonium hydroxide
and the resulting 5'-protecting group is removed with 3%
trichloroacetic acid.   The L-enantiomeric
oligonucleotide is desalted and further purified by

20   elution down an OPEC Cartridge (Clonetech) with
deionized water. Electrophoresis on a 12%
polyacrylamide gel is used to further purify the L-
enantiomeric oligonucleotide.   The L-DNA band is
visualized by ultraviolet light shadowing.   It is cut

25   out, minced, and extracted with a buffer comprising 10mM
Tris HCl and 1mM EDTA at pH 7.5 at 4°C for 24 hours.
The gel pieces are then removed by centrifugation, and
the L-DNA is purified through a spun column of Sephadex
G-25.   The concentration of L-enantiomeric

30   oligonucleotide is estimated using absorbance at 260 nm.

(2b) Synthesis of 5'-H2N-L-d(I-QI-I)-3'-Teg   (SEQ ID NO:
35)
          This L-enantiomeric oligonucleotide is prepared on

35   an Applied Biosystems oligonucleotide synthesizer by the
trityl-off protocol otherwise used for D-enantiomers as

directed by the equipment manufacturer but modified to
use L-2-deoxynucleotide phosphoramidite reagent
precursors (i.e., 5'-dimethoxytrityl L-cytidine-3'-O-
phosphoramidite, 5'-dimethoxytrityl L-adenosine-3'-O-
phosphoramidite, 5'-dimethoxytrityl L-guanosine-3'-O-
phosphoramidite, and 5'-dimethoxytrityl L-thymidine-3'-
O-phosphoramidite as described above). Clonetech's 6
carbon monomethoxytrityl AminoModifier (N-MMT-C6-
AminoModifier) is used as the precursor of the 5'-amine
group. The TEG group is used as the terminal group at
the 3'-end. The base protecting group and solid support
are removed with ammonium hydroxide and the L-
enantiomeric oligonucleotide is further purified by
polyacrylamide gel electrophoresis.

(2c) Synthesis of 5'-Trityl-S-L-d(I-Q_I-I)-3'-NH_2

The L-enantiomeric oligonucleotide sequence of
Example 2a minus the Teg group is prepared on an Applied
Biosystems oligonucleotide synthesizer by the trityl-on
protocol as directed by the equipment manufacturer using
L-2-deoxynucleotide phosphoramidite reagent precursors
(5'-dimethoxytrityl L-cytidine-3'-O-phosphoramidite, 5'-
dimethoxytrityl L-adenosine-3'-O-phosphoramidite, 5'-
dimethoxytrityl L-guanosine-3'-O-phosphoramidite, and
5'-dimethoxytrityl L-thymidine-3'-O-phosphoramidite as
described above). Clonetech's Uni-link Amino Modifier
is used as the precursor to the 3'-amine group.
Clonetech's C6-ThioModifier is used as the precursor to
the 5'-thiol group (which is herein referred to
cryptically as 5-S) added to the sequence in place of
TEG in Example 2a. Following synthesis of the whole L-
enantiomeric oligonucleotide, the base protecting groups
and solid support are removed with ammonium hydroxide.
The L-enantiomeric oligonucleotide is desalted and
further purified by elution down an OPEC Cartridge
(Clonetech) with deionized water. The concentration of

L-enantiomeric oligonucleotide is estimated using absorbance at 260 nm.

5   (2d) Preparation of fluorescent 5'Teg-L-d(I-Q_I-I)-3'-NH-Cy5.18

A sample (30 nmoles) of 5'-Teg-L-d(I-Q_I-I)-3'-NH$_2$, prepared according to Example 2a, is evaporated to dryness and redissolved in 500 mL 0.1 M bicarbonate buffer at pH 9 using a vortex mixer. This sample is
10   then added to a vial containing the dried succinimidyl ester of the dye Cy5.18 (Biological Detection Systems; Pittsburg PA). After thorough mixing the reaction is allowed to proceed at room temperature for one hour with frequent mixing. The product, 5'-Teg-L-d(I-Q_I-I)-3'-NH-
15   Cy5.18, is purified by elution from a Sepahadex G-25 column with distilled water.

Example 3
(3a) Synthesis of cI diamine, 5'-NH$_2$-L-d(cI)-3'-NH$_2$
20   An L-enantiomeric oligonucleotide, L-d(cI), containing the L-enantiomeric nucleotide sequence 5'X-L-d(TTAGCTTCTCCGTCCATAAYT)-3'(SEQ ID NO: 38) complementary to the L-enantiomeric oligonucleotide I is prepared on an Applied Biosystems oligonucleotide synthesizer as
25   outlined in Example 2a. The 3'- (Y) and 5'- (X) amine-containing groups are incorporated as directed by the equipment manufacturer using Uni-link Amino Modifier (Clonetech) for the precursor to the 3'-amine group, and Clonetech's 6 carbon monomethoxytrityl AminoModifier (N-
30   MMT-C6-AminoModifier: Catalog # 5202) as precursor to the 5'-amine group. After final deblocking and removal from the solid support, the protecting groups are removed with ammonium hydroxide, and the amine-functionalized L-enantiomeric oligonucleotide is
35   purified by elution down an OPEC Cartridge (Clonetech) with deionized water. The L-enantiomeric

oligonucleotide is further purified by polyacrylamide
gel electrophoresis or reverse-phase HPLC. The
concentration of L-enantiomeric oligonucleotide is
estimated using absorbance at 260 nm.

5

(3b) Synthesis of I diamine, 5'-NH2-L-d(I)-3'-NH2
       An L-enantiomeric oligonucleotide, I, containing
the L-enantiomeric nucleotide sequence 5'X-L-
d(TTATGGACGGAGAAGCTAAYT)-3'(SEQ ID NO: 39) is prepared

10   on an Applied Biosystems oligonucleotide synthesizer as
outlined in Example 3a. The 3'- (Y) and 5'- (X) amine-
containing groups are incorporated as directed by the
equipment manufacturer using Uni-link Amino Modifier
(Clonetech) for the precursor to the 3'-amine group, and

15   Clonetech's 6 carbon monomethoxytrityl AminoModifier (N-
MMT-C6-AminoModifier: Catalog # 5202) as the precursor
to the 5'-amine group. After final deblocking and
cleavage from the solid support, the protecting groups
are removed with ammonium hydroxide, and the amine-

20   functionalized L-enantiomeric oligonucleotide is
purified by elution down an OPEC Cartridge (Clonetech)
with deionized water. The L-enantiomeric
oligonucleotide is further purified by polyacrylamide
gel electrophoresis or reverse-phase HPLC. The

25   concentration of L-enantiomeric oligonucleotide is
estimated using absorbance at 260 nm.

(3c) Synthesis of L-d(cI)
       An L-enantiomeric oligonucleotide, cI, containing

30   the L-enantiomeric nucleotide sequence 5'-L-
d(TTAGCTTCTCCGTCCATAA)-3' (SEQ ID NO: 23) complementary
to (I) is prepared on an Applied Biosystems
oligonucleotide synthesizer as outlined in Example 2a.
After final deblocking and cleavage from the solid

35   support, the protecting groups are removed with ammonium
hydroxide. The L-enantiomeric oligonucleotide is

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purified by elution down an OPEC Cartridge (Clonetech)
with deionized water. The L-enantiomeric
oligonucleotide is further purified by polyacrylamide
gel electrophoresis. The concentration of L-
5   enantiomeric oligonucleotide is estimated using
absorbance at 260 nm.

(3d) Synthesis of L-d(I)
An L-enantiomeric oligonucleotide, I, containing
10   the L-enantiomeric nucleotide sequence 5'-L-
d(TTATGGACGGAGAAGCTAA)-3' (SEQ ID NO: 8) is prepared on
an Applied Biosystems oligonucleotide synthesizer as
outlined in Example 3c. After final deblocking and
cleavage from the solid support, the protecting groups
15   are removed with ammonium hydroxide, and the L-
enantiomeric oligonucleotide is purified by elution down
an OPEC Cartridge (Clonetech) with deionized water. The
L-enantiomeric oligonucleotide is further purified by
polyacrylamide gel electrophoresis. The concentration
20   of L-enantiomeric oligonucleotide is estimated using
absorbance at 260 nm.

(3e) Preparation of fluorescent cI diamine, 5'-NH$_2$-L-
d(cI)-3'-NH$_2$, and fluorescent I diamine, L-d(I)
25   A sample (30 nmoles) of 5'-NH$_2$-L-d(cI)-3'-NH$_2$,
prepared according to Example 3a, is evaporated to
dryness and redissolved in 500 mL 0.1 M bicarbonate
buffer at pH 9 by vortexing. This sample is then added
to a vial containing the dried N-hydroxysuccinimidyl
30   ester of the dye Cy5.18 (Biological Detection Systems;
Pittsburg PA). After thorough vortexing the reaction is
allowed to proceed at room temperature for one hour with
frequent mixing.   CY5.18 labeled 5'-NH$_2$-L-d(cI)-3'-NH$_2$
is purified by elution from a Sepahadex G-25 column with
35   distilled water.

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A sample of Cy5.18 labeled I diamine, 5'-NH2-L-d(I)-3'-NH2 of Example 3b is prepared in the same manner.

5   Example 4
(4a) Annealing of L-d(cI) to 5'-Teg-L-d(I-QI-I)-3'-NH2
A sample of 5'-Teg-L-d(I-QI-I)-3'-NH2 (2 mmoles), prepared according to Example 2a, is mixed with aliquots containing increasing amounts of L-d(cI) prepared by the
10   method of Example 3c, [from 100:5 to 100:200 of L-d(I-QI-I):L-d(cI)], in 6SSC buffer at room temperature. The mixtures are loaded into a sample cuvette and analysed by UV light (260 nm) in a Cary 13 instrument while the cuvette temperature is ramped up from 30°C to 90°C and
15   then back down to 30°C. The presence of binary complexes (L-d(cI):5'-Teg-L-d(I-QI-I)-3'-NH2 and/or 5'-Teg-L-d(I-QI-I)-3'-NH2:L-d(cI)) are indicated at concentrations of L-d(cI) below equimolar with respect to 5'-Teg-L-d(I-QI-I)-3'-NH2. At higher concentrations
20   of L-d(cI), a ternary complex (L-d(cI):5'-Teg-L-d(I-QI-I)-3'-NH2:L-d(cI)) can be observed.

(4b) Hybridization of TMT-cI-TMT to L-d(I-QI-I)
Similar experiments using L-d(cI)-TMT (see Example
25   6a below) demonstrate the annealing of TMT-L-d(cI)-TMT to 5'-Teg-L-d(I-QI-I)-3'-NH2. A ternary complex is formed.

Example 5
30   Preparation of ^{35}S-labeled TMT-NCS
A suspension of about 40 mmoles of TMT amine (PCT US91/08253) in 650 mL methanol is stirred at room temperature and deionized water is added dropwise (about 70 mL) until a clear pale yellow solution develops. The
35   solution is cooled in an ice bath to 10°C and about 60 mmoles ^{35}S-thiophosgene is added dropwise over about 3

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minutes. A precipate of TMT isothiocyanate forms and
the solution is stirred continuously for a further 2.5
hours. The solution and precipitate are concentrated to
near dryness on a rotovap under reduced pressure (~

5   15mm Hg) at room temperature. The near-dry solid is
diluted further with 750 mL of methanol and is stirred
until the solids appear homogeneous. The solid TMT
isothiocyanate (TMT-NCS) is then collected by filtration
and is rinsed repeatedly with methanol. The product is

10   dried overnight in a vacuum chamber.

Example 6
(6a) Preparation 5'-H_2N-L-d(cI)-3'-NH_2 conjugated to a
      chelating agent, TMT-NCS, to form 5'-TMT-L-d(cI)-

15   3'-TMT and preparation 5'-H_2N-L-d(I)-3'-NH_2
      conjugated to a chelating agent, TMT-NCS, to form
      5'-TMT-L-d(I)-3'-TMT.

      To 300 nmoles of one of the L-enantiomeric
oligonucleotides, either 5'-H_2N-L-d(cI)-3'-NH_2 solution

20   of Example 3a or 5'-H_2N-L-d(I)-3'-NH_2 solution of
Example 3b, in 500 microL of 1.0 M carbonate/bicarbonate
buffer at pH 9.0 is added 12 mg of TMT isothiocyanate
(PCT US91/08253). The reaction mixture is vortex mixed
and kept at 37°C for 2 hours and at room temperature for

25   overnight. The resulting reaction mixture is quenched
with ethanolamine (15 microM) and the product is
purified by Sephadex G-25 column chromatography using
deionized water as the eluting solvent.

      The number of TMT's per molecule of L-enantiomeric

30   oligonucleotide diamine is quantified by an asssay using
the time resolved fluorescence of Europium metal
chelated to the TMT.

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(6b) General procedure for labelling of Ing-1-TMT, 5'-
     TMT-L-d(cI)-3'-TMT, and 5'-TMT-L-d(I)-3'-TMT
     conjugates with fluorescent metals.

Binding of lanthanides such as europium (3^+) to
chelating agents that contain an aromatic moiety held
close to the co-ordination sphere can lead to
"sensitized" fluorescence wherein light is absorbed
through the aromatic system and the energy is
transferred to the metal. The metal then produces
emissions characterized by a very large Stokes shift and
fluorescence lifetimes of up to several seconds. The
fluorescence at 615 nm is measured at a time-delay of
400 microseconds after an excitation pulse at 340 nm.
This time delay is useful for high sensitivity
measurements since short lived background fluorescence
is eliminated and up to a 1,500 fold enhancement in
sensitivity over normal Eu-fluorescence is achievable.

In this method, a known amount of Ing-1-TMT, 5'-
TMT-L-d(cI)-3'-TMT, or 5'-TMT-L-d(I)-3'-TMT conjugate
is titrated with increasing amounts of added EuCl3 in an
aqueous buffer. Thus, one microliter of a solution
containing 1-30 picomoles of the conjugate is added, in
duplicate, to wells in a Costar EIA/RIA 96-well plate
containing a precalculated amount of Tris.HCl buffer (pH
7.4). The buffer volume is derived by subtracting from
99 the volume in microliters of aqueous EuCl3 (typically
10^{-4} M to 10^{-6} M in Tris.HCl buffer). The total volume
in each well is thereby fixed at 100 microliters.
Aqueous EuCl3 is then added to the buffered solution of
the conjugate. The plate is then covered and shaken at
low speed for one hour. The time resolved fluorescence
is then measured using a Delfia 1232 time-resolved
fluorometer (Wallac Inc.) and the data are analyzed. It
is found that each conjugated TMT molecule chelates one
Europium ion and that both 5'-TMT-L-d(cI)-3'-TMT and 5'-

TMT-L-d(I)-3'-TMT conjugates bind two Europium ions per
molecule of conjugate.

(6c)   Preparation of ^{35}S-labeled TMT-L-d(cI)-TMT from 5'-
       H$_2$N-L-d(cI)-3'-NH$_2$ and ^{35}S-labeled TMT-NCS.

^{35}S-labeled TMT-NCS (prepared as in Example 5) is
substituted for the 12 mg of TMT isothiocyanate (PCT
US91/08253) in the method of Example 6a and the reaction
carried out as described above. The number of TMT's per
molecule of 5'-H$_2$N-L-d(cI)-3'-NH$_2$ is quantified by
counting the TMT-L-d(cI)-TMT product in a liquid
scintillation counter optimized to detect ^{35}S. From a
knowledge of the concentration of derivatized 5'-H$_2$N-L-
d(cI)-3'-NH$_2$ and the specific activity of ^{35}S-
thiophosgene, the number of TMT molecules per 5'-H$_2$N-L-
d(cI)-3'-NH$_2$ diamine may be calculated.

Example 7
(7a)   Preparation of a Yttrium (^{90}Y) radiolabeled-L-
       enantiomeric oligonucleotides, ^{90}Y-TMT-L-d(cI)-TMT-
       ^{90}Y and ^{90}Y-TMT-L-d(I)-TMT-^{90}Y
       A solution of the L-enantiomeric oligonucleotide
TMT conjugates, either (TMT-L-d(cI)-TMT) from Example 6a
or TMT-L-d(I)-TMT from Example 6a, in deionized water at
room temperature is treated with a solution of ^{90}YCl$_3$
(>500 Ci/mg; from Amersham Corp.) in 0.5 M sodium
acetate buffer at pH 6.0 to a specific activity of 0.1
Ci/pmole for one hour at room temperature. The labeling
efficiency is determined by removing 1.0 microliter of
the sample and spotting it on to a Gelman ITLC-SG
strip. The strip is developed in a glass beaker
containing 0.1 M sodium citrate at pH 6.0 for a few
minutes until the solvent front reached three-quarters
of the way to the top of the paper. The strip is then
inserted into a System 200 Imaging Scanner (Bioscan)

which had been optimized for ^{90}Y and which is controlled
by a Compaq 386/20e computer. In this system unbound
^{90}Y migrates at the solvent front. The TMT's of the L-
enantiomeric oligonucleotide TMT conjugates chelate in
5   excess of 98 % of the added radioactivity.

(7b) Hybridization of radiolabeled ^{90}Y-TMT-L-d(cI)-TMT-
       ^{90}Y or of ^{90}Y-TMT-L-d(I)-TMT-^{90}Y to 5'-Teg-L-d(I-
       Q_I-I)-3'-NH$_2$

10   A 5 pmole sample of ^{90}Y-TMT-L-d(cI)-TMT-90Y from
Example 7(a) is mixed with increasing amounts (0.375 to
12 pmoles) of 5'-Teg-L-d(I-Q_I-I)-3'-NH$_2$ from Example 2a
in PBS at 37°C for one hour. A 5 mL aliquot from each
of these hybridizations is removed, mixed with SDS-
15   containing buffer, and run on a 12% PAGE gel.
Autoradiographs of the gels reveal that 5'-Teg-L-d(I-Q_I-
I)-3'-NH$_2$ is capable of binding to 2 molecules of cI.
          Similar procedures using non-complementary, ^{90}Y-TMT-
L-d(I)-TMT-^{90}Y from Example 7(a) fail to show
20   hybridization to 5'-Teg-L-d(I-Q_I-I)-3'-NH$_2$.

(7c) Administration of ^{90}Y-TMT-L-d(cI)-TMT-^{90}Y to Nude
       Mice
          TMT-L-d(cI)-TMT labeled with ^{90}Y to a specific
25   activity of 28 mCi/28 mg is injected into a 25 g nude
mouse bearing a subcutaneous tumor in its right flank.
At time intervals after injection, blood samples are
taken from the tail and counted for ^{90}Y radioactivity in
a liquid scintillation counter. The results reveal that
30   more than 95% of the injected dose of ^{90}Y is removed
from the blood stream in the first 30 minutes following
injection.   After 2 hours following injection the
radioactivity in the blood levels off and a minute
fraction (< 0.01% of injected dose) continues to
35   circulate during the next 22 hours. These data
demonstrate that in the absence of a hybridizing site

recognizing the L-d(cI) sequence, radiolabeled TMT-L-d(cI)-TMT is lost quickly from the circulation.

Example 8

5   (8a) Preparation of Antibody-Maleimide (Ab-M) using
              Sulfo-SMCC and ING-1; (ING-1-Maleimide).
              A Sulfo-SMCC solution (108 nmoles) in phosphate
       buffered saline (PBS) is added to a sample of a chimeric
       antibody (ING-1; 18 nmoles) solution in phosphate buffer
10   (pH 7). The resulting mixture is allowed to stand for 30
       minutes with occasional mixing at room temperature. The
       reaction is stopped with 60 nmoles basic tris buffer.
       The reaction mixture is diluted with phosphate buffered
       saline, added to a prewashed PD-10 column, and eluted
15   with PBS to afford ING-1-maleimide. This material is
       stored on ice until use.

       (8b) Preparation of mercaptoalkyl-Antibody (Ab-SH) from
              ING-1 and 2-iminothiolane; (ING-1-SH)
20   A sample of a chimeric antibody (ING-1; 5 nmoles)
       solution in 0.1 M carbonate buffer (pH 8.8) is mixed
       with 200 nmoles of an aqueous solution of 2-
       iminothiolane. The resulting mixture is allowed to
       stand for 30 min with occasional mixing at room
25   temperature. The reaction mixture is diluted with
       phosphate buffed saline, added to a prewashed PD-10
       column (Pharmacia), and eluted with PBS to afford
       mercaptoalkyl-ING-1. This material is stored on ice
       until use.
30

Example 9

       (9a) Preparation of a mercaptoalkyl-L-d(I-Q_I-I) using 2-
              iminothiolane; 5'-Teg-L-d(I-Q_I-I)-3'-SH
              A sample of a solution of 5'-Teg-L-d(I-Q_I-I)-3'-NH$_2$
35   (30 nmoles) in water is mixed with 1 M carbonate buffer
       (pH 9) to give a final buffer concentration of 890 mM.

Into the buffered L-DNA is added 12 mmoles of an aqueous
solution of 2-iminothiolane hydrochloride. These
reactants are vortex mixed and kept at 37°C for 30
minutes. The reaction mixture is quenched by the

5   addition of 12 mmoles of ethanolamine, diluted with
phosphate buffed saline, added to a prewashed NAP-25
column (Pharmacia), and eluted with PBS to afford 5'Teg-
L-d(I-Q_I-I)-3'-NH-C(N=H$_2^+$)CH$_2$CH$_2$CH$_2$SH (as the
hydrochloride Cl⁻). For use in conjugation to a

10   maleimide-derivatized antibody (Ab-M), the product is
eluted from the column directly into the antibody
solution. Otherwise, the mercaptoalkyl-L-d(I-Q_I-I) is
stored on ice until use.

15   (9b) Preparation of a mercaptoalkyl-L-d(I-Q_I-I) using 2-
            iminothiolane; 5'-HS-L-d(I-Q_I-I)-3'-Teg
            A sample of 5'-H$_2$N-L-d(I-Q_I-I)-3'-Teg (30 nmoles)
       is treated as in Example 9a to afford 5'-HS-
       CH$_2$CH$_2$CH$_2$C(=NH$_2^+$)-HN-L-d(I-Q_I-I)-Teg-3' (Cl⁻).

20
       (9c) Preparation of an L-d(I-Q_I-I)-Maleimide using
            Sulfo-SMCC; 5'-Teg-L-d(I-Q_I-I)-3'-Maleimide
            An aqueous solution containing 20 nmoles of 5'-Teg-
       L-d(I-Q_I-I)-3'-NH$_2$ (prepared as in Example 2a) is

25   diluted into phosphate buffed saline. Sulfo-SMCC (100
       nmoles) in PBS is added and the resulting mixture is
       allowed to stand for 30 min with occasional mixing at
       room temperature. The reaction mixture is diluted with
       phosphate buffed saline, added to a prewashed PD-10

30   column, and eluted with PBS to afford 5'-Teg-L-d(I-Q_I-
       I)-3'-Maleimide. This material is stored on ice until
       use.

(9d) Preparation of an L-d(I-Q$_I$-I)-Maleimide; 5'-
    Maleimide-L-d(I-Q$_I$-I)-3'-Teg.

5'-H$_2$N-L-d(I-Q$_I$-I)-3'-Teg is reacted with sulfo-
SMCC in the same manner as 5'-Teg-L-d(I-Q$_I$-I)-3'-NH$_2$ in
9c to afford 5'-Maleimide-L-d(I-Q$_I$-I)-3'Teg.

(9e) Preparation of 3'-mercapto-L-d(I-Q$_I$-I) using SATA

An aqueous solution containing 50 nmoles of 5'-Teg-
L-d(I-Q$_I$-I)-3'-NH$_2$ (prepared as in Example 2a) is
diluted into PBS and 500 nmoles of SATA (in DMSO) is
added. After mixing and standing at room temperature
for 60 min, the reaction mixture is diluted with PBS and
eluted from a NAP-10 column with PBS to afford 5'-Teg-L-
d(I-Q$_I$-I)-3'NH-CO-CH$_2$-S-CO-CH$_3$. The sulfhydryl group of
the acetylthioacetylated L-enantiomeric oligonucleotide
is deacylated by the addition of 30 mL of a pH 7.5
solution containing 100 mM sodium phosphate, 25 mM EDTA,
and 500 mM NH$_2$OH. The reaction is allowed to proceed
for two hours at room temperature after which time the
material is passed down a NAP-5 column using PBS for the
elution. The product, 5'-Teg-L-d(I-Q$_I$-I)-3'NH-CO-CH$_2$-
SH, is used immediately to obviate oxidative
dimerization.

(9f) Preparation of 5'mercapto-L-d(I-Q$_I$-I) using SATA

H$_2$N-5'-L-d(I-Q$_I$-I)-3'-Teg is reacted with SATA in
the same manner as 5'-Teg-L-d(I-Q$_I$-I)-3'-NH$_2$ in Example
9e to afford 5'-HS-CH$_2$-OC-NH-L-d(I-Q$_I$-I)-3'-Teg.

Example 10
(10a) Conjugation of 5'Teg-L-d(I-Q$_I$-I)-3'-NH-
    C(=NH$_2$$^+$)CH$_2CH_2CH_2$SH to Ab-M; ING-1-Maleimido-3'-S-
    L-d(I-Q$_I$-I)-5'-Teg

A sample (108 nmoles) of 5'-Teg-L-d(I-Q$_I$-I)-3'-NH-
C(=NH$_2$$^+$)CH$_2CH_2CH_2$SH (prepared according to Example 9a)
is eluted with PBS from a NAP-25 column directly into a

solution of Ab-M (maleimide-derivatized ING-1; 18
nmoles) from Example 8a. After mixing, the reaction is
allowed to proceed for 20 hours at 4°C. The reactants
are then loaded into Centricon-100® ultrafiltration
concentration devices (Amicon) which are then
centrifuged at 1000 g for 25 minutes. The sample
containing the product is resuspended in fresh PBS, and
ultrafiltration, concentration by centrifugation and
resuspension are repeated a further 3 times until the
ratio of optical densities at 260 nm and 280 nm is
constant. The final product is ING-1-Maleimido-S-
$(CH_2)_3$-C$(=NH_2{}^+)$-NH-3'-L-d$(I-Q_I-I)$-5'-Teg.

(10b) Conjugation of 5'-HSCH$_2$CH$_2$CH$_2$ $(NH_2{}^+=)$C-NH-L-d$(I-Q_I-$
        I)-Teg-3' to Antibody-Maleimide; ING-1-Maleimide-
        5'-S-L-d$(I-Q_I-I)$-Teg-3'
      A sample (108 nmoles) of 5'-HSCH$_2$CH$_2$CH$_2$ $(NH_2{}^+=)$C-NH-
L-d$(I-Q_I-I)$-Teg-3' prepared according to Example 9b is
conjugated to 18 nmoles of maleimide-derivatized ING-1
as in Example 8a to give ING-1-Maleimide-5'-S-$(CH_2)_3$-
C$(=NH_2{}^+)$-NH-L-d$(I-Q_I-I)$-3'-Teg.

(10c) Assays on the ING-1-Maleimide-S-L-d$(I-Q_I-I)$
        conjugates
      The optical density of each of the ING-1-Maleimide-
S-$(CH_2)_3$-C$(=NH)$NH-L-d$(I-Q_I-I)$ samples of example 10(a)
and 10(b) is examined in a spectrophotometer at 260 nm
and 280 nm. The ratio of optical densities at these two
wavelengths is calculated, and, by using known
extinction coefficients for the antibody and for the L-
enantiomeric oligonucleotide (approximate molecular
weight 16,500) at each of these wavelengths, the number
of L-enantiomeric oligonucleotide molecules, L-d$(I-Q_I-$
I), is estimated to be between 1 and 2 L-d$(I-Q_I-I)$ per
antibody in the sample.

The concentration of ING-1 in a conjugate solution
is determined by the BioRad protein assay using bovine
immunoglobulin as the protein standard. These data
agreed well with the antibody concentrations determined

5   by examination of the optical density of the conjugate
at 280 nm once it has been corrected for absorbance due
to the conjugated L-d(I-Q_I-I). Both these sets of data
are further confirmed by subjecting the antibody-L-d(I-
Q_I-I) conjugates to acid digestion and amino acid

10   analysis.
Antibody-L-d(I-Q_I-I) conjugates are examined for
their ability to bind to antigens on the surface of a
human tumor cell line to which the antibody is raised.
The immunoreactivity of the conjugates is compared by

15   flow cytometry with a standard preparation of the
antibody before being subjected to modification and
conjugation to L-d(I-Q_I-I). Target HT29 cells (a human
adenocarcinoma cell line: ATTC) are grown to confluency
in tissue culture flasks using McCoy's media

20   supplemented with 10% fetal calf serum. The cells are
harvested by scraping the flask walls with a cell
scraper. Cells from many separate flasks are pooled,
centrifuged to a pellet, resuspended at 5 x 10^5/mL in a
solution of ice-cold 50 mM sodium phosphate with 150 mM

25   sodium chloride buffer pH 7.4 (PBS) supplemented with
0.1% bovine serum albumin (Sigma) and 0.02% sodium azide
(Flow buffer). The cells are washed in this same buffer
and then counted. An antibody standard curve is
constructed by diluting a stock solution of ING-1 with

30   an irrelevant (non binding), isotype-matched control
antibody (human IgG_1) to give a number of samples
ranging in ING-1 content from 10% to 100%. A standard
curve is made in flow buffer so that each sample
contains 1.0 mg antibody protein per mL. Samples from

35   the standard curve and unknowns are then incubated with
5 x 10^5 HT29 cells at 4°C for 1 hour. After extensive

washing to remove unbound antibody, the cells are
resuspended in 100 mL flow buffer and incubated at 4°C
for 1 hour with goat-anti-human antibody labeled with
fluorescene isothiocyanate (FITC). After further

5   washing in flow buffer the samples are analyzed by flow
cytometry on a Coulter EPICS 753 flow cytometer.
Fluorescene from Fluorescene isothiocyanite (FITC) and
propidium iodide (PI) is excited using the 488 nm
emission line of an argon laser. The output is set at

10   500 mW in light regulation mode. Single cells are
identified by 90 degree and forward angle light scatter.
Analysis windows are applied to these parameters to
separate single cells from aggregates and cell debris.
Fluorescence from FITC and propidium are separated with

15   a 550 nm long pass dichroic filter and collected through
a 530 nm band pass filter (for FITC), and a 635 nm band
pass filter (for PI). Light scatter parameters are
collected as integrated pulses and fluorescence is
collected as log integrated pulses. Dead cells are

20   excluded from the assay by placing an analysis window on
cells negative for PI uptake. The mean fluorescence per
sample (weighted average from 2500 cells) is calculated
for each histogram. FITC calibration beads are analysed
in each experiment to establish a fluorescence standard

25   curve. The average fluorescence intensity for each
sample is then expressed as the average FITC equivalents
per cell. Immunoreactivity is calculated by comparing
the average fluorescence intensity of the unknown sample
with values from the standard curve. From the

30   immunoreactivity assay, ING-1-Maleimide-S-$(CH_2)_3$-
$C(=NH_2^+)$-NH-3'L-d$(I-Q_I-I)$-5'-Teg is approximately 2/3 as
immunoreactive as the ING-1 standard and ING-1-
Maleimide-S-$(CH_2)_3$-$C(=NH_2^+)$-NH-5'-L-d$(I-Q_I-I)$-3'-Teg is
approximately 4/5 as immunoreactive. In a separate set

35   of experiments, immunoreactivity of ING-1-L-d$(I-Q_I-I)$
(3'-conjugate) and ING-1-L-d$(I-Q_I-I)$ (5'-conjugate) are

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determined.   Samples of these conjugates are also
subjected to electrophoresis on Novex 6% polyacrylamide
gels using SDS-containing buffers in order to estimate
their apparent molecular weight and the degree of

5   heterogeneity of the preparation.   Using standards of
known molecular weight run on the same gel, a standard
curve is constructed of the distance travelled (Rf)
versus the log of the molecular weight.   From this
standard curve the relative molecular weights of the

10   bands associated with each conjugate preparation are
determined.   The SDS PAGE gels of ING-1-L-d(I-Q_I-I) and
ING-1 antibody demonstrate that the molecular weight of
the ING-1-L-d(I-Q_I-I) conjugates are higher than that of
the antibody ING-1 alone.

15

(10d)   Conjugation of Teg-5'-L-d(I-Q_I-I)-3'-Maleimide to
        mercaptoalkyl-Antibody, Ab-SH;   ING-1-NH-CO-CH$_2$-S-
        Maleimido-3'-L-d(I-Q_I-I)-5'Teg
        20 nmoles of Teg-5'-L-d(I-Q_I-I)-3'-Maleimide (from

20   Example 9c) are reacted with 5 nmoles of mercaptoalkyl-
ING-1 (Ab-SH from Example 8b) in PBS pH 7.   After
mixing, the reaction is allowed to continue at 4°C for
16 hours to afford ING-1-NH-CO-CH$_2$-S-Maleimido-3'-L-d(I-
Q_I-I)-5'-Teg.   The reaction mixture is loaded into a

25   Centricon-100® ultrafiltration concentration device
which is then centrifuged at 1,000 g for 25 minutes.
The sample is resuspended in fresh PBS and this sequence
of concentration by centrifugation is repeated a further
3 times until the ratio of optical densities at 260 nm

30   and 280 nm is constant.
        Maleimide-5'-L-d(I-Q_I-I)-3'-Teg is reacted with
mercaptoalkyl-ING-1 (Ab-SH) in the same way to afford
ING-1-NH-CO-CH$_2$-S-Maleimide-5'-L-d(I-Q_I-I)-3'Teg.

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(10e) Conjugation of Teg-5'-L-d(I-Q$_I$-I)-3'-NH-C(=O)-CH$_2$-
       SH to Antibody-Maleimide, Ab-M.

      A 6 nmole sample of ING-1-Maleimide (Ab-M from
Example 8a) in PBS is reacted with 40 nmoles of Teg-5'-
L-d(I-Q$_I$-I)-3'-NH-CO-CH$_2$-SH (from Example 9e) at 4°C for
16 hours. The reactants are diluted with PBS and eluted
in PBS from a PD-10 column to afford ING-1-Maleimido-S-
CH$_2$-C(=O)-NH-3'-L-d(I-Q$_I$-I)-5'Teg. The product is
concentrated in a Centricon-300® device by
centrifugation at 1000g for 25 minutes. The sample is
resuspended in fresh PBS and concentration by
centrifugation. This process is repeated a further 3
times until the ratio of optical densities at 260 nm and
280 nm is constant.

      HS-CH$_2$-(O=)C-NH-5'-L-d(I-Q$_I$-I)-3'-Teg is reacted
with ING-1-Maleimide in the same way to afford ING-1-
Maleimido-S-CH$_2$-(O=)C-NH-5'-L-d(I-Q$_I$-I)-3'-Teg. The
optical density of these samples is examined in a
spectrophotometer at 260 nm and 280 nm. The ratio of
optical densities at these two wavelengths is then
calculated. By using known extinction coefficients for
the antibody and for the L-enantiomeric oligonucleotide
(approx molecular weight 16,500) at each of these
wavelengths, the number of L-enantiomeric
oligonucleotide molecules per antibody is estimated.

(10f) Conjugation of 5'-Trityl-(S)-L-d(I-Q$_I$-I)-3'-NH$_2$ to
       Antibody-Maleimide, Ab-M.

      A sample (10 nmoles) of 5'-Trityl(S)-L-d(I-Q$_I$-I)-
3'-NH$_2$ from Example 2c is diluted into PBS and a
solution of silver nitrate in water is added to a final
concentration of 85 mM. After vortexing, the reaction
is allowed to proceed at room temperature for 30
minutes. A precipitate forms which is centifuged to the
bottom of the tube. The clear supernatant which
contains the L-enantiomeric oligonucleotide with a 5'

terminal thiol group and without the 5' trityl group
(HS-5'-L-d(I-Q$_I$-I)-3'-NH$_2$) is kept at 4°C until use.

5   A 6 nmole sample of ING-1-Maleimide (from Example
8a) in PBS is reacted with 40 nmoles of HS-5'-L-d(I-Q$_I$-
I)-3'-Teg at 4°C for 16 hours. The reactants are
diluted with PBS and eluted in 4 mL from a pre-washed
Econopac 106-DG column (BioRad) to afford ING-1-
Maleimide-S-5'-L-d(I-Q$_I$-I)-3'-Teg. The product is
concentrated in a Centricon-300® concentration device by
10   centrifugation at 1000 g for 25 minutes. The sample is
resuspended in fresh PBS and concentration by
centrifugation is repeated a further 3 times until the
ratio of optical densities at 260 nm and 280 nm is
constant.

15   The optical density of these samples are examined
in a spectrophotometer at 260 nm and 280 nm. The ratio
of optical densities at these two wavelengths is
calculated, and by using known extinction coefficients
for the antibody and for the L-enantiomeric
20   oligonucleotide (approximate molecular weight=16500) at
each of these wavelengths, the number of L-enantiomeric
oligonucleotide molecules per antibody is estimated.

Example 11
25   (11a)   Annealing of L-d(cI) to ING-1-L-d(I-Q$_I$-I)
A sample of ING-1-Maleimide-S-(CH$_2$)$_3$-C(=NH$_2^+$)-NH-
3'-L-d(I-Q$_I$-I)-5'-Teg (24 pmoles) from Example 10a and a
sample of ING-1-Maleimide-S-(CH$_2$)$_3$-C(=NH$_2^+$)-NH-5'L-d(I-
Q$_I$-I)-3'-Teg (25 pmoles) from Example 10d are mixed in
30   separate cuvettes with 16-fold excess of L-d(cI) from
Example 3c in 50 mM PBS containing 1.0 mM EDTA and 100
mM NaCl, pH 7.2, at room temperature. The cuvettes are
cooled to 20°C, loaded into a Cary 13 instrument, and
the absorbance is analysed by UV light (260 nm) while
35   the cuvette temperature is ramped up from 20°C to 80°C
and then back down to 20°C at a rate of 0.5°C/min.

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Analysis of the data reveals that cI is able to
hydridize to both the 3'- and the 5'- conjugates to form
a ternary complex with each.   Similar experiments
using TMT-L-d(cI)-TMT (from Example 6) demonstrate the
5   annealing of TMT-L-d(cI)-TMT to ING-1-Maleimido-S-
$(CH_2)_3$-C$(=NH_2^+)$-NH-3'-L-d(I-Q$_I$-I)-5'-Teg.

(11b) Hybridization of ING-1-L-d(I-Q$_I$-I) to either ^{90}Y-
TMT-L-d(cI)-TMT-^{90}Y or ^{90}Y-TMT-L-d(I)-TMT-^{90}Y in
10   phosphate buffer and human serum
A solution of ING-1-Maleimido-S-$(CH_2)_3$-C$(=NH_2^+)$-NH-
3'-L-d(I-Q$_I$-I)-5'-Teg (4 ul; 1 mg antibody/ml from
Example 10a) and 50 microL ^{90}Y-TMT-L-d(cI)-TMT-^{90}Y
solution from Example 9a are mixed with freshly prepared
15   human serum (200 mL) or PBS (200 mL; pH7.2) and
incubated at 37°C for 2 hours. Aliquots are then
subjected to SDS PAGE electrophoresis on an 8 to 16%
gel. The gels are examined by both autoradiography and
on a phosphoimager system to show that ^{90}Y-TMT-L-d(cI)-
20   TMT-^{90}Y is able to hybridize with ING-1-L-d(I-Q$_I$-I) in
human serum as well as phosphate buffer solution (pH
7.2). Samples are left at room temperature for 14 days
in PBS and in serum and run on 8 to 16% SDS-PAGE to give
similar patterns to each other and to gels incubated at
25   37°C for 2 hours. This indicates that the conjugates
are stable in serum for up to two weeks. ^{90}Y-TMT-L-d(I)-
TMT-^{90}Y from Example 9a fails to show hybridization to
ING-1-L-d(I-Q$_I$-I) at any time.

30   (11c) Hybridization of ^{90}Y-TMT-L-d(cI)-TMT-^{90}Y to ING-1-
L-d(I-Q$_I$-I)
A solution of ING-1-Maleimido-S-$(CH_2)_3$-C$(=NH_2^+)$-NH-
3'-L-d(I-Q$_I$-I)-5'-Teg (4ul; 1mg/ml from Example 10a) and
50 uL ^{90}Y-TMT-L-d(cI)-TMT-^{90}Y (from Example 9a) are
35   mixed in PBS (200 ul; pH 7.2) and incubated at 37°C for
60 minutes. Aliquots of these mixtures are then

subjected to SDS PAGE electrophoresis on an 8 to 16%
gel. The gels are autoradiographed on a phosphoimager
system to show that ^{90}Y-TMT-L-d(cI)-TMT-^{90}Y is able to
hybridize with ING-1-L-d(I-Q_I-I).

5

(11d) Western blot to detect TMT on TMT-L-d(cI)-TMT
       hybridized with ING-1-L-d(I-Q_I-I).

A sample of L-d(I-Q_I-I) from Example 2a or of ING-
1-Maleimido-3'-S-(CH$_2$)$_3$-C(=NH$_2^+$)-NH-L-d(I-Q_I-I)-5'-Teg

10   from Example 10a is separately mixed with the L-
enantiomeric oligonucleotide TMT conjugate TMT-L-d(cI)-
TMT from Example 6. The reaction mixture of each is
kept on ice for 10 minutes. Aliquots of each reaction
mixture are mixed with SDS buffer and loaded onto two

15   duplicate 8 to 16% polyacrylamide gels. The gels are
subjected to electrophoresis at a constant voltage for 2
hours. One gel is electroblotted onto nitrocellulose
paper using CAPS buffer for 20 minutes according to the
manufacturer's protocol (Hoefer semi-dry transfer

20   method). After washing thrice with a solution of 0.05%
Tween 20 in PBS, the gel is blocked with a solution of
3% BSA in PBS at room temperature for 1 hour. Following
further washing with Tween/PBS, the gel is overlaid with
a solution of a murine anti-TMT antibody (10mg/ml in

25   PBS/Tween) and left overnight at room temperature. The
western blot is developed using a goat anti-mouse IgG
antibody conjugated to horseradish peroxidase (BioRad
Western blot kit) and peroxidase substrate. The blot
demonstrates that the TMT-L-d(cI)-TMT can be detected

30   via the TMT's as being hybridized to bands that
contained either L-d(I-Q_I-I) or ING-1-L-d(I-Q_I-I).

The second gel is stained with ethidium bromide
(5mg/ml in distilled water). Examination of the stained
gel under UV light again demonstrates hybrization.

35

Example 12

Preparation of Ab-TMT by direct conjugation (ING-1/TMT)

TMT-NCS (or a suitable derivative thereof) can be conjugated to an antibody molecule to yield an antibody-TMT conjugate molecule that displays the ability to bind to a target antigen recognized by the antibody variable region. Such a conjugate molecule can be used to deliver metal ions that are chelated by the TMT moiety in order to localize and/or treat the tumor that is targeted by such an immunoconjugate. In one preferred embodiment, the antibody is selected such that it has a broad reactivity with an antigen molecule expressed on tumor cells, thereby providing an antibody-TMT conjugate that can deliver radionuclides to the tumors for therapeutic or diagnostic purposes. The chimeric antibody, ING-1, (International patent publication WO 90/02569) consists of a murine variable region and a human immunoglobulin constant region. The antibody is produced by culturing a mouse myeloma cell line expressing the chimeric antibody essentially as described in the above-referenced publication. After purification, ING-1 is used at a concentration of 5.0 mg/mL in 50 mM sodium acetate and 150 mM sodium chloride buffered at pH 5.6.

The conjugation of ING-1 to TMT-NCS is achieved by first adding 1.0 M carbonate plus 150 mM sodium chloride buffer, pH 9.3, to ING-1 until the antibody solution reaches a pH of 9.0. A sample of that ING-1 solution containing 5 mg of protein is then pipetted into an acid washed, conical, glass reaction vial. A solution of TMT-NCS is prepared by dissolving 100 mg in 10 mL of 1.0 M carbonate plus 150 mM sodium chloride buffer, pH 9.0. The conjugation reaction is started by the addition of 96.5 mL of the TMT-NCS solution to the antibody to give a 4-fold molar excess of TMT-NCS over ING-1. The solution is stirred briefly to mix the

reactants and then left in the dark at room temperature.
After 16 hours, the ING-1/TMT conjugate is separated
from unconjugated TMT by applying the reaction mixture
to a PD10 chromatography column which has been pre-

5   washed and equilibriated with 50 mM sodium acetate in
150 mM sodium chloride buffer, pH 5.6. The pure
conjugate is eluted from the column with 2.5 mL of that
same buffer.

10   Example 13
Analytical tests on the ING-1/TMT conjugates.
(13a) Analysis of Chelator to Antibody Ratio
The protein concentrations of ING-1 in the
conjugate solutions are determined by the BioRad protein

15   assay using bovine immunoglobulin as the protein
standard.
In order to calculate the number of functional TMT
molecules per antibody, ING-1/TMT is reacted with a
solution of Europium chloride until saturation of the

20   metal-binding capacity of the TMT occurs. A 0.375 mg
aliquot of the ING-1/TMT in 2.5 ml in 0.05 M Tris HCl
buffer pH 7.5 is pipetted into a 5 ml quartz cuvette. A
20 mM Europium chloride (Europium chloride hexahydrate;
Aldrich) solution in 0.05 M Tris HCl buffer pH 7.5 is

25   prepared. An aliquot (50 mL) of this Europium chloride
solution is added to the cuvette containing ING-1/TMT
and the resulting solution is slowly stirred on a
magnetic stirrer at room temperature for 10 min using a
small magnetic stir bar placed in the cuvette. The

30   fluorescence of the metal-ING-1/TMT complex is
determined in a Perkin Elmer LS 50 spectrofluorometer
using an excitation wavelength of 340 nm (10 nm slit
width). The fluorescent emission is monitored at 618 nm
using a 10 nm slit width and a 430 nm cutoff filter.

35   The above procedure is repeated and fluorescent readings
are made after each addition. Aliquots of Europium

chloride are added until the increase in fluorescence
intensity is less than 5% of the preceding reading. A
dilution correction is applied to the fluorescence
intensity measured at each mole ratio to compensate for

5   the change in volume of the test solution. Since each
chelating site on the ING-1/TMT conjugate binds one
Europium ion, and since Europium ion has to be in a
chelate site for fluorescence to occur, this method
allows the number of functional chelation sites to be

10   quantitated.
       Using this method, the ratio of TMT molecules per
molecule of antibody is in the range from 0.3:1 to 2:1.

       (13b) ING-1/TMT Immunoreactivity assay by ELISA

15   The antigen to which the antibody, ING-1, binds is
prepared from LS174T or HT 29 cells (available from
American Type Tissue Collection, ATTC) by scraping
confluent monolayers of cells from the walls of culture
flasks with a cell scraper. The cells from many flasks

20   are combined and a sample is taken and counted to
estimate the total number of cells harvested. At all
times the cells are kept on ice. Following
centrifugation of the cells at 1500 rpm for 10 minutes
at 4°C, the cells are washed once in 25 mL ice-cold 50

25   mM sodium phosphate buffer supplemented with 150 mM
sodium chloride, pH 7.4 (PBS), pelleted under the same
conditions and transfered in 10 mL PBS to an ice-cold
glass mortar. The cells are homogenized at 4°C using a
motor-driven pestle and then centrifuged at 3000 x g for

30   5 minutes. The antigen-rich supernatant is removed from
the other cell debris and subjected to further
centrifugation at 100,000 x g for one hour at 4°C. The
pellet (antigen fraction) from this final step is
suspended in 100 mL of PBS for every million cells

35   harvested. Following an estimate of the protein
concentration (BioRad BCA protein assay using bovine

immunoglobulin as the protein standard) the antigen is
stored at -20°C until use. Each well of a 96-well
Costar microtiter plates is coated with antigen by
adding 100 mL/well of cell lysate (10 mg/ml) prepared as
5   above. The microtiter plates are allowed to dry
overnight in a 37°C incubator. After washing the plates
five times with 0.05% Tween-20 (Sigma) they are blotted
dry. The wells of each plate are blocked by adding 125
mL/well of a 1% BSA (bovine serum albumin, Sigma A-7906)
10   solution in PBS and incubated for 1 hour at room
temperature. The plates are washed five times with
0.05% Tween-20. Samples (50 mL/well in duplicate) of
ING-1/TMT conjugates and standard ING-1 antibody
solutions are prepared at a range of concentrations in
15   1% BSA in PBS. Biotinylated ING-1 (1.0 mg/mL in 0.1%
BSA) is added to each well (50mL/well) and the plates
are then incubated for 2 hours at room temperature.
Following five washes with 0.05% Tween-20, the plates
are blotted dry and incubated at room temperature for
20   one hour with dilute (1:2000 in 0.1% BSA) streptavidin-
alkaline phosphatase (Tago; #6567). After a further
five washes, color is developed in each well upon the
addition of 100 mL per well of phosphatase substrate
reagent (Sigma). After one hour at room temperature,
25   the color is read using a 405 nm filter in a Titertek
Multiscan microplate reader.

        When tested by this procedure, the immunoconjugates
of ING-1 with TMT are found to have immunoreactivity
comparable to native ING-1.
30

        (13c) ING-1/TMT Immunoreactivity Assay by Flow Cytometry
        Target HT29 cells are grown to confluency in tissue
culture flasks using McCoy's media supplemented with 10%
fetal calf serum. The cells are harvested by scraping
35   the flask walls with a cell scraper. Cells from many
separate flasks are pooled, centrifuged to a pellet,

resuspended at 5 x 10^5/mL in a solution of ice-cold 50
mM sodium phosphate with 150 mM sodium chloride buffer
pH 7.4 (PBS) supplemented with 0.1% bovine serum albumin
(Sigma) and 0.02% sodium azide (Flow buffer). The cells

5   are washed in this same buffer and then counted. An
antibody standard curve is constructed by diluting ING-1
with an irrelevant (non binding), isotype-matched
control antibody (human IgG1) to give a number of
samples ranging in ING-1 content from 10% to 100%. The

10   standard curve is made in flow buffer so that each
sample contains 1.0 mg protein per mL. Samples from the
standard curve and unknowns are then incubated with 5 x
10^5 HT29 cells at 4°C for 1 hour. After extensive
washing to remove unbound antibody, the cells are

15   resuspended in 100 mL flow buffer and incubated at 4°C
for 1 hour with goat-anti-human antibody labeled with
fluorescene isothiocyanate (FITC). After further
washing in flow buffer the samples are analyzed by flow
cytometry on a Coulter EPICS 753 flow cytometer.

20   Fluorescene FITC and propidium iodide (PI) are excited
using the 488 nm emission line of an argon laser. The
output is set at 500 mw in light regulation mode.
Single cells are identified by 90 degree and forward
angle light scatter. Analysis windows are applied to

25   these parameters to separate single cells from
aggregates and cell debris. Fluorescence from FITC and
propidium are separated with a 550 nm long pass dichroic
filter and collected through a 530 nm band pass filter
(for FITC), and a 635 nm band pass filter (for PI).

30   Light scatter parameters are collected as integrated
pulses and fluorescence is collected as log integrated
pulses. Dead cells are excluded from the assay by
placing an analysis window on cells negative for PI
uptake. The mean fluorescence per sample (weighted

35   average from 2500 cells) is calculated for each
histogram. FITC calibration beads are analysed in each

experiment to establish a standard curve. The average
fluorescence intensity for each sample is then expressed
as the average FITC equivalents per cell.

5   Immunoreactivity is calculated by comparing the average
fluorescence intensity of the unknown sample with values
from the standard curve. Samples of ING-1/TMT have
immunoreactivity values comparable to the native ING-1
antibody by this method.

10   (13d) Determination of aggregate formation by size-
exclusion HPLC.

A 30 cm x 7.5 mm TSK-G3000SW size-exclusion HPLC
column (Supelco) fitted with a guard column of the same
material is equilibrated with 12 column volumes of 10 mM

15   sodium phosphate buffer pH 6.0 supplemented with 150 mM
sodium chloride using a Waters 600E HPLC system with a
flow rate of 1.0 mL per minute at 400-600 PSI. A sample
(25 mL) of BioRad gel filtration protein standards is
injected on to the column. The retention time of each

20   standard is monitored by a Waters 490 UV detector set at
280 nm. Following the recovery of the final standard
from the column, it is washed with a further 10 volumes
of 10 mM sodium phosphate buffer, pH 6.0, supplemented
with 150 mM sodium chloride. Samples (50mL) of either

25   native ING-1 antibody or ING-1/TMT at 200 mg/mL are
injected onto the column and their retention times are
recorded. From the areas of the retained peaks and the
retention time, the amount of aggregated material in the
ING-1/TMT sample is calculated.

30   By this method the native ING-1 antibody has a
retention time of 9.1 minutes. ING-1/TMT has a major
peak also at 9.1 minutes but a minor peak, attributable
to aggregates, can sometimes be seen at 7.3 minutes. By
comparison of the peak areas, the aggregate peak is less

35   than 5% of the total.

(13e) Radiolabeling of ING-1/TMT with ^{90}Y.

A volume of radioactive Yttrium chloride (^{90}Y in
0.04 M hydrochloric acid at a specific activity of >500
Ci/mg; Amersham-Mediphysics) is neutralized using two
5   volumes of 0.5 M sodium acetate pH 6.0. The neutralized
^{90}Y (1.0 mCi) is added to 1.0 mL of ING-1/TMT (1 mg/mL)
in 50 mM sodium acetate buffer containing 150 mM sodium
chloride at pH 5.6. The labelling is allowed to proceed
for one hour and then the reaction mixture is loaded
10   onto a PD-10 chromatography column which has been pre-
washed and equilibrated in a buffer containing 50 mM
sodium phosphate with 150 mM sodium chloride pH 7.4
(PBS). The sample is eluted from the column with 1.5 mL
of PBS. Fractions of radiolabeled ING-1/TMT (0.5 mL)
15   are collected, assayed for radioactivity, and pooled.
The labeling efficiency is determined by removing 1.0 mL
of the sample and spotting it on to a Gelman ITLC-SG
strip. The strip is developed in a glass beaker
containing 0.1 M sodium citrate, pH 6.0, for a few
20   minutes until the solvent front has reached three-
quaters of the way to the top of the paper. The strip
is inserted into a System 200 Imaging Scanner (Bioscan)
which has been optimized for ^{90}Y and is controlled by a
Compaq 386/20e computer. In this system free ^{90}Y
25   migrates at the solvent front while the ING-1/TMT/^{90}Y
remains at the origin.

Using this system more than 98% of the total ^{90}Y
radioactivity is always found associated with ING-1/TMT
at the origin.
30

(13f) Labeling ING-1/TMT with fluorescent metals.

Binding of lanthanides such as europium (3+) to
chelating agents that contain an aromatic moiety held
close to the co-ordination sphere can lead to
35   "sensitized" fluorescence wherein light is absorbed
through the aromatic system and the energy is transfered

to the metal. The metal then produces emissions
characterized by a very large Stokes shift and
fluorescence lifetimes of up to several seconds. A 0.5
mg aliquot of the ING-1/TMT in 2.5 mL in 0.05 M Tris HCl
5   buffer pH 7.5 is pipetted into a 4 mL conical reaction
vial containing a small stirring bar. A 250 mM europium
chloride (europium chloride hexahydrate: Aldrich)
solution in 0.05 M Tris HCl buffer pH 7.5 is prepared.
An aliquot (50 mL) of this europium chloride solution is
10   added to the reaction vial containing ING-1/TMT, and the
resulting solution is stirred very slowly on a magnetic
stirrer at room temperature. The labelling is allowed
to proceed for one hour and then the reaction mixture is
loaded on to a PD-10 chromatography column which had
15   been pre-washed and equilibrated in a buffer containing
10 mM sodium phosphate and 150 mM sodium chloride at pH
6.0 (PBS). The sample is eluted from the column with
3.5 mL of PBS. The fluorescence of a 50 mL sample of
the metal-ING-1/TMT complex is determined in a Perkin
20   Elmer LS 50 spectrofluorometer using an excitation
wavelength of 340 nm (10 nm slit width). The
fluorescent emission is recorded at 618 nm using a 10 nm
slit width and a 430 nm cutoff filter. Each functional
chelating site on the ING-1/TMT conjugate binds one
25   europium ion. Using this method, an average of between
0.1 and 3 fluorescent europium ions are bound per
molecule of antibody in solution.

Example 14
30   (14a) Flow Cytometry of binding of fluorescent L-d(cI)
to cells treated with ING-1-L-d(I-Q_I-I).
ING-1-Maleimide-S-$(CH_2)_3$-C$(=NH_2^+)$-NH-3'-L-d(I-Q_I-
I)-5'Teg is prepared as in Example 10a. ING-1-Maleimide-
S-$(CH_2)_3$-C$(=NH_2^+)$-NH-5'-L-d(I-Q_I-I)-3'-Teg is prepared
35   as in Example 10b. Fluorescently labeled CY5.18-L-d(I-
Q_I-I) is prepared as in Example 2d. Fluorescently

labeled CY5.18-L-d(cI) and CY5.18-L-d(I) are prepared as
in Example 3e. Flow cytometry is carried out
essentially as described in Example 12c except that the
fluorescent dye CY5.18 is used in place of FITC. HT-29

5   cells (0.5×10^6) are incubated on ice for 30 min with 1
mg each of the ING-1-L-d(I-Q$_I$-I) samples. The cells are
washed twice with flow buffer and pelleted at 1400 rpm
for 5 minutes between washes. Next the cells in each
sample are incubated with 5 mg CY5.18-L-d(cI) or CY5.18-

10   L-d(I) for 3 hours on ice. Some cells are incubated
with CY5.18-L-d(cI) and CY5.18-L-d(I) alone. After
extensive washing with flow buffer, the cells are
subjected to analysis on a fluorescence activated cell
sorter. CY5-18 calibration beads are analysed to

15   establish a standard curve of relative fluorescence
intensity versus CY5-18 concentration. The mean
fluorescence per sample (weighted average from 2500
cells) is calculated for each histogram. The average
fluorescence intensity for each sample is then expressed

20   as the average CY5-18 equivalents per cell. Identical
experiments are carried out in which the medium used for
incubation of the cells with the components is 100%
fetal calf serum in place of flow buffer.

         The time taken to maximize hybridization of CY5-18-

25   L-d(cI) to ING-1-Maleimide-S-(CH$_2$)3-C(=NH$_2$+)-NH-3'-L-
d(I-Q$_I$-I)-5'-Teg on the surface of HT-29 cells is about
3 hours. There is no difference between flow buffer and
100% FCS in the degree of hybridization in each
preparation suggesting that the end-capped L-

30   enantiomeric oligonucleotide strands are stable to
nuclease digestion. Large amounts of CY5.18-L-d(cI) can
hybridize to both conjugates bound to the surface of
cells. There is relatively little non-specifc binding
to the cells either by the fluorescent L-enantiomeric

35   oligonucleotides themselves or by hybridization of CY5-
18-L-d(I-Q$_I$-I) to the conjugates.

(14b) Binding of ^{90}Y-TMT-L-d(cI)-TMT-^{90}Y to HT-29 cells
       treated with ING-1-L-d(I-Q$_I$-I).

TMT-L-d(cI)-TMT is radiolabeled to a specific
activity of 0.1 mCi/pmole as described in Example 7.
ING-1-TMT-^{90}Y is prepared as in Example 13e. ING-1-
Maleimide-S-(CH$_2$)3-C(=NH$_2$$^+$)-NH-3'-L-d(I-Q$_I$-I)-5'-Teg is
prepared as in Example 10a. Three tubes each containing
1 x 10^5 HT-29 cells in DMEM medium supplemented with 10%
fetal calf serum are prepared and kept at 4°C
throughout. Using matched protein concentrations in all
tubes to ensure that the amount of added antibody is
equal, the tubes are treated as shown in Table 1 below.

Table 1

Time (min)	Sample 1	Sample 2	Sample 3
0	+ING-1-L-d(I-Q$_I$-I)	0	0
60	Wash x2	Wash x2	Wash x2
90	+L-d(cI)-(TMT^{90}Y)2	ING-1-TMT^{90}Y	Prehybridized ING-1-L-d-(I-Q$_I$-I) with L-d(cI)-(TMT^{90}Y)2
150	Wash x2	Wash x2	Wash x2
180	Centrifuge cells and count pellet radioactivity	Centrifuge cells and count pellet radioactivity	Centrifuge cells and count pellet radioactivity

Both sample 1 and sample 3 show higher
radioactivity associated with the cell pellet than does
sample 2 (directly labeled ING-1-TMT-^{90}Y) suggesting
that a delivery system consisting of 2 separate

(14c) Binding of Europium labeled ING-1/TMT and TMT-L-
       d(cI)-TMT conjugates to HT-29 cells and HT-29
       cells treated with ING-1-L-d(I-Q_I-I), respectively

5   The TMT-L-d(cI)-TMT conjugate is labeled with
Europium ions as described in Example 6b and ING-1/TMT
is labeled with Europium ions as described in Example
13f. Standard curves are created for both conjugates in
concentration ranges of 100 picomoles/100 microL to 6
10   femtomoles/100 microL. HT-29 cells are grown to
confluence in McCoys media containing 10% FCS and 50
microgram/ml of gentamyacin. The cells are washed with
phosphate buffered saline, and 5 ml of Trypsin Versene
is added. The HT-29 cells are then incubated at 37°C in
15   5% CO_2 for 15 minutes, complete media (5 ml) is added,
and the cells are removed and washed in PBS. The HT-29
cells are then blocked with 10 micrograms of sheared
salmon sperm (natural D-enantiomer) DNA per 10^6 cells at
4°C for 30 minutes, washed in PBS and used in the
20   hybridization assay as follows. 5 x 10^5 HT-29 cells are
added to a 100 microL working dilution of Ing-1/TMT or
Ing-1-L-d(I-Q_I-I) and the mixture is incubated for 30
minutes on ice. The cells are washed twice in 2 ml of a
wash buffer (PBS + 0.1% BSA + 0.01% NaN_3) at 1400 RPM
25   for 5 minutes. A working dilution of TMT-L-d(cI)-TMT
(100 microL) is then added to the appropriate tubes and
the reaction mixture is incubated on ice for 3 hours
followed by washing twice in 2 mL of wash buffer (PBS +
0.1% BSA + 0.01% NaN_3) at 1400 RPM for 5 minutes.
30   Each binding study is done in triplicate. The cell
suspensions after binding and hybridization as above are
kept at 4°C in test tubes until use (10 minutes to one
hour). The Europium fluorescence is measured in a
Delfia 1232 time-resolved fluorimeter by aliquoting four
35   100 microL portions into separate wells in a Costar
EIA/RIA 96-well plate from each tube after vortexing.

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The results are processed as described in Exampl 15f.
The counts per seconds (cps) of the Eu-TMT-L-d(cI)-TMT-
Eu treated cells are considered as background and are
subtracted from the cps data from the hybridization

5   experiments (Eu-TMT-L-d(cI)-TMT-Eu/Ing-1-L-d(I-Q$_I$-I)).
This result and the cps data from the Ing-1/TMT-Eu
binding experiment are translated as picomoles of bound
TMT molecules per cell from the individual standard
curves created independently.

10   The results are described as fluorescence counts
per second (cps) or number (#) of picomoles per 10^5
cells. ING-1/TMT-Eu and ING-conjugates: 0.25 microgram
or 1.65 picomoles; Eu-TMT-L-d(cI)-TMT-Eu: 100 ng or 15
picomoles per 5 X 10^5 cells. For the same degree of

15   modification of ING-1 by TMT and by L-d(I-Q$_I$-I) to form
ING-1-TMT and ING-1-L-d(I-Q$_I$-I), respectively, an
increase or amplification in fluorescence is seem in the
latter when Eu is added to form ING-1/TMT-Eu from ING-1-
TMT versus when Eu-TMT-L-d(cI)-TMT-Eu is added to ING-1-

20   L-d(I-Q$_I$-I) to form the hybrid, respectively.

         The invention has been described in detail with
particular reference to certain preferred embodiments
thereof, but it will be understood that variations and

25   modifications can be effected within the spirit and
scope of the invention.

.SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT: Black, Christopher D.V.
              Snow, Robert A.

  (ii) TITLE OF INVENTION: TUMOR TARGETING WITH L-ENANTIOMERIC
        OLIGONUCLEOTIDE CONJUGATES OF IMMUNOREAGENTS AND OF
        CHELATED RADIONUCLIDES

 (iii) NUMBER OF SEQUENCES: 39

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   (v) COMPUTER READABLE FORM:
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      (B) COMPUTER: IBM PC compatible
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      (B) FILING DATE:
      (C) CLASSIFICATION:

 (viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

  (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 12 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

 (ii) MOLECULE TYPE: DNA (genomic)

 (ix) FEATURE:
      (A) NAME/KEY: misc_feature
      (B) LOCATION: 1..12
      (D) OTHER INFORMATION: /note= "Each nucleotide is the
         L-enantiomeric form."

 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

/11

TTATGGACGG AG   12

(2) INFORMATION FOR SEQ ID NO:2:

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            (A) LENGTH: 13 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: DNA (genomic)

       (ix) FEATURE:
            (A) NAME/KEY: misc_feature
            (B) LOCATION: 1..13
            (D) OTHER INFORMATION: /note= "Each nucleotide is the
                L-enantiomeric form."

       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTATGGACGG AGA   13

(2) INFORMATION FOR SEQ ID NO:3:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 14 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: DNA (genomic)

       (ix) FEATURE:
            (A) NAME/KEY: misc_feature
            (B) LOCATION: 1..14
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       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTATGGACGG AGAA   14

(2) INFORMATION FOR SEQ ID NO:4:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 15 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: DNA (genomic)

       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TTATGGACGG AGAAG   15

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(2) INFORMATION FOR SEQ ID NO:5:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 16 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
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        (B) LOCATION: 1..16
        (D) OTHER INFORMATION: /note= "Each nucleotide is the
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    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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(2) INFORMATION FOR SEQ ID NO:6:

    (i) SEQUENCE CHARACTERISTICS:
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        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
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        (B) LOCATION: 1..17
        (D) OTHER INFORMATION: /note= "Each nucleotide is the
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    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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(2) INFORMATION FOR SEQ ID NO:7:

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        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

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        (B) LOCATION: 1..18
        (D) OTHER INFORMATION: /note= "Each nucleotide is the
            L-enantiomeric form."

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TTATGGACGG AGAAGCTA   18

(2) INFORMATION FOR SEQ ID NO:8:

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        (A) LENGTH: 19 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
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        (B) LOCATION: 1..19
        (D) OTHER INFORMATION: /note= "Each nucleotide is the
            L-enantiomeric form."

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TTATGGACGG AGAAGCTAA   19

(2) INFORMATION FOR SEQ ID NO:9:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 12 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

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    (ix) FEATURE:
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        (B) LOCATION: 1..12
        (D) OTHER INFORMATION: /note= "Each nucleotide is the
            L-enantiomeric form."

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGGAGAAGCT AA   12

(2) INFORMATION FOR SEQ ID NO:10:

    (i) SEQUENCE CHARACTERISTICS:
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        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
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        (B) LOCATION: 1..13

        (D)   THER INFORMATION: /note= "Each nucleotide is the
              L-enantiomeric form."

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ACGGAGAAGC TAA   13

(2) INFORMATION FOR SEQ ID NO:11:

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          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: DNA (genomic)

     (ix) FEATURE:
          (A) NAME/KEY: misc_feature
          (B) LOCATION: 1..14
          (D) OTHER INFORMATION: /note= "Each nucleotide is the
              L-enantiomeric form."

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GACGGAGAAG CTAA   14

(2) INFORMATION FOR SEQ ID NO:12:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 15 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
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     (ix) FEATURE:
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          (B) LOCATION: 1..15
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     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

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(2) INFORMATION FOR SEQ ID NO:13:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 16 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
     (A) NAME/KEY: misc_feature
     (B) LOCATION: 1..16
     (D) OTHER INFORMATION: /note= "Each nucleotide is the
         L-enantiomeric form."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TGGACGGAGA AGCTAA   16

(2) INFORMATION FOR SEQ ID NO:14:

  (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 17 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: DNA (genomic)

  (ix) FEATURE:
     (A) NAME/KEY: misc_feature
     (B) LOCATION: 1..17
     (D) OTHER INFORMATION: /note= "Each nucleotide is the
         L-enantiomeric form."

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ATGGACGGAG AAGCTAA   17

(2) INFORMATION FOR SEQ ID NO:15:

  (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 18 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: DNA (genomic)

  (ix) FEATURE:
     (A) NAME/KEY: misc_feature
     (B) LOCATION: 1..18
     (D) OTHER INFORMATION: /note= "Each nucleotide is the
         L-enantiomeric form."

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TATGGACGGA GAAGCTAA   18

(2) INFORMATION FOR SEQ ID NO:16:

  (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 12 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
         (A) NAME/KEY: misc_feature
         (B) LOCATION: 1..12
         (D) OTHER INFORMATION: /note= "Each nucleotide is the
             L-enantiomeric form."

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TTAGCTTCTC CG   12

(2) INFORMATION FOR SEQ ID NO:17:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 13 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
         (A) NAME/KEY: misc_feature
         (B) LOCATION: 1..13
         (D) OTHER INFORMATION: /note= "Each nucleotide is the
             L-enantiomeric form."

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TTAGCTTCTC CGT   13

(2) INFORMATION FOR SEQ ID NO:18:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 14 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
         (A) NAME/KEY: misc_feature
         (B) LOCATION: 1..14
         (D) OTHER INFORMATION: /note= "Each nucleotide is the
             L-enantiomeric form."

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TTAGCTTCTC CGTC   14

(2) INFORMATION FOR SEQ ID NO:19:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 15 base pairs

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        (B)  TYPE: nucleic acid
        (C)  STRANDEDNESS: single
        (D)  TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)


   (ix) FEATURE:
        (A)  NAME/KEY: misc_feature
        (B)  LOCATION: 1..15
        (D)  OTHER INFORMATION: /note= "Each nucleotide is the
             L-enantiomeric form."


   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TTAGCTTCTC CGTCC                                                      15

(2) INFORMATION FOR SEQ ID NO:20:

   (i) SEQUENCE CHARACTERISTICS:
        (A)  LENGTH: 16 base pairs
        (B)  TYPE: nucleic acid
        (C)  STRANDEDNESS: single
        (D)  TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)


   (ix) FEATURE:
        (A)  NAME/KEY: misc_feature
        (B)  LOCATION: 1..16
        (D)  OTHER INFORMATION: /note= "Each nucleotide is the
             L-enantiomeric form."


   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TTAGCTTCTC CGTCCA                                                     16

(2) INFORMATION FOR SEQ ID NO:21:

   (i) SEQUENCE CHARACTERISTICS:
        (A)  LENGTH: 17 base pairs
        (B)  TYPE: nucleic acid
        (C)  STRANDEDNESS: single
        (D)  TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)


   (ix) FEATURE:
        (A)  NAME/KEY: misc_feature
        (B)  LOCATION: 1..17
        (D)  OTHER INFORMATION: /note= "Each nucleotide is the
             L-enantiomeric form."


   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TTAGCTTCTC CGTCCAT                                                    17
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(2) INFORMATI N FOR SEQ ID NO:22:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 18 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)

   (ix) FEATURE:
        (A) NAME/KEY: misc_feature
        (B) LOCATION: 1..18
        (D) OTHER INFORMATION: /note= "Each nucleotide is the
           L-enantiomeric form."

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TTAGCTTCTC CGTCCATA   18

(2) INFORMATION FOR SEQ ID NO:23:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 19 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)

   (ix) FEATURE:
        (A) NAME/KEY: misc_feature
        (B) LOCATION: 1..19
        (D) OTHER INFORMATION: /note= "Each nucleotide is the
           L-enantiomeric form."

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TTAGCTTCTC CGTCCATAA   19

(2) INFORMATION FOR SEQ ID NO:24:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 12 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)

   (ix) FEATURE:
        (A) NAME/KEY: misc_feature
        (B) LOCATION: 1..12
        (D) OTHER INFORMATION: /note= "Each nucleotide is the
           L-enantiomeric form."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CTCCGTCCAT AA   12

(2) INFORMATION FOR SEQ ID NO:25:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 13 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
       (A) NAME/KEY: misc_feature
       (B) LOCATION: 1..13
       (D) OTHER INFORMATION: /note= "Each nucleotide is the
          L-enantiomeric form."

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TCTCCGTCCA TAA   13

(2) INFORMATION FOR SEQ ID NO:26:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 14 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
       (A) NAME/KEY: misc_feature
       (B) LOCATION:1..14
       (D) OTHER INFORMATION: /note= "Each nucleotide is the
          L-enantiomeric form."

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TTCTCCGTCC ATAA   14

(2) INFORMATION FOR SEQ ID NO:27:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 15 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
       (A) NAME/KEY: misc_feature
       (B) LOCATION: 1..15

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        (D) OTHER INFORMATION: /note= "Each nucleotide is the
            L-enantiomeric form."

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CTTCTCCGTC CATAA   15

(2) INFORMATION FOR SEQ ID NO:28:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 16 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
        (A) NAME/KEY: misc_feature
        (B) LOCATION: 1..16
        (D) OTHER INFORMATION: /note= "Each nucleotide is the
            L-enantiomeric form."

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GCTTCTCCGT CCATAA   16

(2) INFORMATION FOR SEQ ID NO:29:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 17 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
        (A) NAME/KEY: misc_feature
        (B) LOCATION: 1..17
        (D) OTHER INFORMATION: /note= "Each nucleotide is the
            L-enantiomeric form."

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

AGCTTCTCCG TCCATAA   17

(2) INFORMATION FOR SEQ ID NO:30:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 18 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

```
(ix) FEATURE:
     (A) NAME/KEY: misc_feature
     (B) LOCATION: 1..18
     (D) OTHER INFORMATION: /note= "Each nucleotide is the
              L-enantiomeric form."


(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:
```

TAGCTTCTCC GTCCATAA   18

(2) INFORMATION FOR SEQ ID NO:31:

```
    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 52 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)


   (ix) FEATURE:
        (A) NAME/KEY: misc_feature
        (B) LOCATION: 1
        (D) OTHER INFORMATION: /note= "N equals Teg."

   (ix) FEATURE:
        (A) NAME/KEY: misc_feature
        (B) LOCATION: 2..50
        (D) OTHER INFORMATION: /note= "Each nucleotide is the
                 L-enantiomeric form."

   (ix) FEATURE:
        (A) NAME/KEY: misc_feature
        (B) LOCATION: 51
        (D) OTHER INFORMATION: /note= "N equals NH2."

   (ix) FEATURE:
        (A) NAME/KEY: misc_feature
        (B) LOCATION: 52
        (D) OTHER INFORMATION: /note= "This nucleotide is the
                 L-enantiomeric form."


   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:
```

NTCTTATGGA CGGAGAAGCT AAACTCTCTT ATGGACGGAG AAGCTAATCT NT   52

(2) INFORMATION FOR SEQ ID NO:32:

```
    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 45 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)



   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:
```

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TCTTATGGAC GGATCCGCTA ATCTTATGGA CGGATCCGCT AATCT   45

(2) INFORMATION FOR SEQ ID NO:33:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 52 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
            (A) NAME/KEY: misc_feature
            (B) LOCATION: 1
            (D) OTHER INFORMATION: /note= "N equals an
                 amine-containing group."

    (ix) FEATURE:
            (A) NAME/KEY: misc_feature
            (B) LOCATION: 51
            (D) OTHER INFORMATION: /note= "N equals an
                 amine-containing group."

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

NTCTTATGGA CGGAGAAGCT AAACTCTCTT ATGGACGGAG AAGCTAATCT NT   52

(2) INFORMATION FOR SEQ ID NO:34:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 52 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
            (A) NAME/KEY: misc_feature
            (B) LOCATION: 1
            (D) OTHER INFORMATION: /note= "N equals an
                 amine-containing group."

    (ix) FEATURE:
            (A) NAME/KEY: misc_feature
            (B) LOCATION: 2..50
            (D) OTHER INFORMATION: /note= "Each nucleotide is the
                 L-enantiomeric form."

    (ix) FEATURE:
            (A) NAME/KEY: misc_feature
            (B) LOCATION: 51
            (D) OTHER INFORMATION: /note= "N equals an
                 amine-containing group."

    (ix) FEATURE:
            (A) NAME/KEY: misc_feature
            (B) LOCATION: 52

            (D) OTHER INFORMATION: /note= "This nucleotide is the
                L-enantiomeric f rm."

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

NTCTTATGGA CGGAGAAGCT AAACTCTCTT ATGGACGGAG AAGCTAATCT NT   52

(2) INFORMATION FOR SEQ ID NO:35:

      (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 52 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA (genomic)

      (ix) FEATURE:
            (A) NAME/KEY: misc_feature
            (B) LOCATION: 1
            (D) OTHER INFORMATION: /note= "N equals H2N."

      (ix) FEATURE:
            (A) NAME/KEY: misc_feature
            (B) LOCATION: 2..50
            (D) OTHER INFORMATION: /note= "Each nucleotide is the
                L-enantiomeric form."

      (ix) FEATURE:
            (A) NAME/KEY: misc_feature
            (B) LOCATION: 51
            (D) OTHER INFORMATION: /note= "N   Teg."

      (ix) FEATURE:
            (A) NAME/KEY: misc_feature
            (B) LOCATION: 52
            (D) OTHER INFORMATION: /note= "This nucleotide is the
                L-enantiomeric form."

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

NTCTTATGGA CGGAGAAGCT AAACTCTCTT ATGGACGGAG AAGCTAATCT NT   52

(2) INFORMATION FOR SEQ ID NO:36:

      (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 50 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA (genomic)

      (ix) FEATURE:
            (A) NAME/KEY: misc_feature
            (B) LOCATION: 1
            (D) OTHER INFORMATION: /note= "N equals H2N."

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(ix) FEATURE:
         (A) NAME/KEY: misc_feature
         (B) LOCATION: 2..50
         (D) OTHER INFORMATION: /note= "Each nucleotide is the
             L-enantiomeric form."


     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

NTCTTATGGA CGGAGAAGCT AAACTCTCTT ATGGACGGAG AAGCTAATCT                    50

(2) INFORMATION FOR SEQ ID NO:37:

     (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 51 base pairs
         (B) TYPE: nucleic acid
         (C) STRANDEDNESS: single
         (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: DNA (genomic)


     (ix) FEATURE:
         (A) NAME/KEY: misc_feature
         (B) LOCATION: 1
         (D) OTHER INFORMATION: /note= "N equals H2N."

     (ix) FEATURE:
         (A) NAME/KEY: misc_feature
         (B) LOCATION: 2..50
         (D) OTHER INFORMATION: /note= "Each nucleotide is the
             L-enantiomeric form."

     (ix) FEATURE:
         (A) NAME/KEY:misc_feature
         (B) LOCATION: 51
         (D) OTHER INFORMATION: /note= "N equals OPO3H2."


     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

NTCTTATGGA CGGAGAAGCT AAACTCTCTT ATGGACGGAG AAGCTAATCT N                  51

(2) INFORMATION FOR SEQ ID NO:38:

     (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 22 base pairs
         (B) TYPE: nucleic acid
         (C) STRANDEDNESS: single
         (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: DNA (genomic)


     (ix) FEATURE:
         (A) NAME/KEY: misc_feature
         (B) LOCATION: 1
         (D) OTHER INFORMATION: /note= "N equals an
             amine-containing group."

     (ix) FEATURE:
         (A) NAME/KEY: misc_feature
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          (B) LOCATION: 2..20
          (D) OTHER INFORMATION: /note= "Each nucleotide is the
              L-enantiomeric form."

     (ix) FEATURE:
          (A) NAME/KEY: misc_feature
          (B) LOCATION: 21
          (D) OTHER INFORMATION: /note= "N equals an
              amine-containing group."

     (ix) FEATURE:
          (A) NAME/KEY: misc_feature
          (B) LOCATION: 22
          (D) OTHER INFORMATION: /note= "This nucleotide is the
              L-enantiomeric form."


     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

NTTAGCTTCT CCGTCCATAA NT                                          22

(2) INFORMATION FOR SEQ ID NO:39:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 22 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: DNA (genomic)


     (ix) FEATURE:
          (A) NAME/KEY: misc_feature
          (B) LOCATION: 1
          (D) OTHER INFORMATION: /note= "N equals an
              amine-containing group."

     (ix) FEATURE:
          (A) NAME/KEY: misc_feature
          (B) LOCATION: 20
          (D) OTHER INFORMATION: /note= "Each nucleotide is the
              L-enantiomeric form."

     (ix) FEATURE:
          (A) NAME/KEY: misc_feature
          (B) LOCATION: 21
          (D) OTHER INFORMATION: /note= "N equals an
              amine-containing group."

     (ix) FEATURE:
          (A) NAME/KEY: misc_feature
          (B) LOCATION: 22
          (D) OTHER INFORMATION: /note= "This nucleotide is the
              L-enantiomeric form."


     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

NTTATGGACG GAGAAGCTAA NT                                          22
```
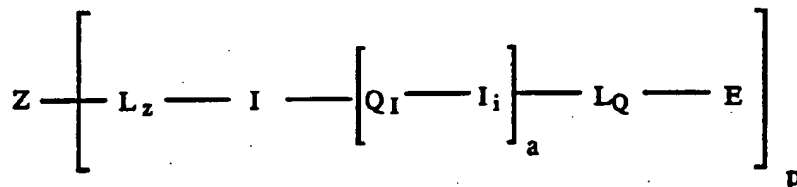
We claim:

1.   A non-radioactive targeting immunoreagent that comprises a tumor antigen recognizing moiety, one or more non-self-associating L-enantiomeric oligonucleotide sequences, and one or more linking groups.

2.   A radioactive targeting immunoreagent that comprises one or more chelating agents, one or more linking groups, one or more radionuclides, and an L-enantiomeric oligonucleotide sequence that is complementary in sequence to and capable of hybridization with one or more fragments of a non-self-associating L-enantiomeric oligonucleotide sequence.

3.   A targeting immunoreagent that comprises moieties represented by the structure IV:

Structure IV

$$Z \left[L_z - I - \left[Q_I - I_i \right]_a - L_Q - E \right]_p$$

wherein:

Z is the residue of an immunoreactive protein;

L_z and L_Q are independently a chemical bond or a linking group;

I is an L-enantiomeric oligonucleotide containing a contiguous sequence of from 12 to about 50 L-enantiomeric nucleotide units wherein said contiguous sequence contains one or more members of a family of homologous contiguous sequences, the individual homologs of said family containing from 12 to about 30 L-enantiomeric nucleotide units, provided that contiguous

sequences of six or more L-enantiomeric nucleotide units
of said L-enantiomeric oligonucleotide do not hybridize
with any other contiguous sequences of six or more
contiguous L-enantiomeric nucleotide units anywhere in
5   structure IV;

Q$_I$ is a spacing group;
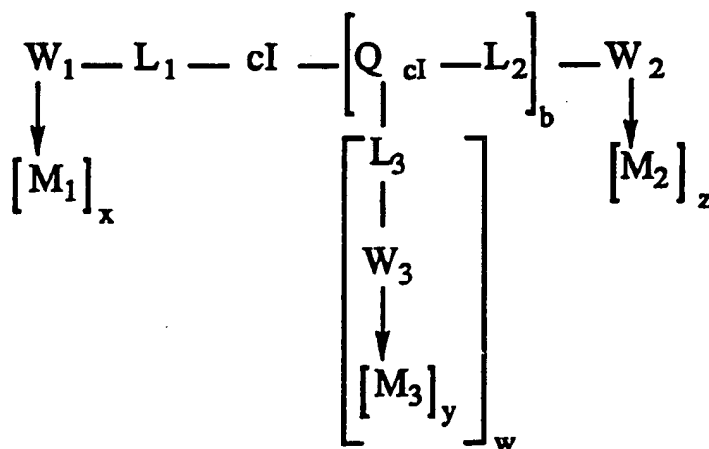
a is 0 or an integer from 1 to about 6;

I$_i$ is an L-enantiomeric oligonucleotide contiguous
sequence of from 12 to about 50 L-enantiomeric
10   nucleotide units, a contiguous sequence therein of which
is identical to a contiguous sequence in I;

E is an end capping group; and

p is an integer from 1 to about 10.

15   4.   A radioactive targeting reagent comprising
moieties represented in the structure V:

Structure V

$$W_1 - L_1 - cI - \left[\underset{\downarrow}{Q} \; cI - L_2 \right]_b - W_2$$

$$\downarrow \qquad\qquad\qquad\qquad\qquad \downarrow$$

$$[M_1]_x \qquad\qquad \left[\begin{array}{c} L_3 \\ | \\ W_3 \\ \downarrow \\ [M_3]_y \end{array} \right]_w \qquad [M_2]_z$$

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wherein:

cI is an L-enantiomeric oligonucleotide contiguous
sequence of from 12 to about 50 L-enantiomeric
25   nucleotide units wherein said contiguous sequence
contains one or more members of a family of homologous

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contiguous sequences, the individual homologs of which family comprise from 12 to about 30 L-enantiomeric nucleotide units; the L-enantiomeric nucleotide sequences of said homologs are complementary to L-enantiomeric nucleotide sequences of members of the set of L-enantiomeric oligonucleotides in a co-administerable targeting immunoreagent; and contiguous sequences of six or more L-enantiomeric nucleotide units of said complementary L-enantiomeric oligonucleotide do not hybridize with any other contiguous sequences of six or more contiguous L-enantiomeric nucleotide units anywhere in structure V;

Q_{CI} is a spacing group;

L_1, L_2, and L_3 are each independently a chemical bond or a linking group;

W_1, W_2, and W_3 are each independently a residue of a chelating group;

M_1, M_2 and M_3 each comprise an element with an oxidation state equal to or greater than +1, and at least one of M_1, M_2 and M_3 is a radionuclide;

x, y, and z are independently zero or one provided that at least one of x, y, or z is one; and

w and b are independently zero or an integer from 1 to about 4.

5.   The reagent of claim 3 wherein a is an integer from one to about 6.

6.   The reagent of claim 3 wherein Z is the residue of an antibody or the residue of an antibody fragment.

7.   The reagent of claim 6 wherein the antibody is selected from ING-1; B72.3; 9.2.27; D612; UJ13A; NRLU-10; 7E11C5; CC49; TNT; PR1A3; B174; C174; B43; and anti-HLB antibodies.

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8. The reagent of claim 3 wherein L_Z or L_Q is a residue of a heterobifunctional cross-linking reagent.

9. The reagent of claim 8 wherein the heterobifunctional cross-linking reagent is selected from the group consisting of sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate, sulfosuccinimidyl (4-iodoacetyl)aminobenzoate, sulfosuccinimidyl 4-(p-maleimidophenyl)butyrate, 2-iminothiolane, and N-succinimidyl S-acetylthioacetate.

10. The reagent of claim 3 wherein L_Z or L_Q is a residue of a modified nucleotide moiety comprising a reactive functional group.

11. The reagent of claim 10 where the reactive functional group is selected from the group consisting of amine groups and sulfhydryl groups.

12. The reagent of claim 3 wherein I and I_i are L-enantiomeric DNA.

13. The reagent of claim 3 wherein I has the sequence shown in SEQ ID NO: 8.

14. The reagent of claim 3 wherein Q_I is the residue of an L-enantiomeric oligonucleotide.

15. The reagent of claim 14 wherein the L-enantiomeric oligonucleotide has the sequence L-d(ACTCTC).

16. The reagent of claim 3 wherein E is the residue of a modified nucleotide group resistant to exonuclease activity.

17.   The reagent of claim 16 wherein the modified
nucleotide comprises a poly(alkylene glycol) phosphate
diester.

5   18.   The reagent of claim 17 wherein the diester is
tetra(ethylene glycol) phosphate diester.

        19.   The reagent of claim 4 wherein cI has the
sequence shown in SEQ ID NO: 23.

10   20.   The reagent of claim 4 wherein Q_I is the
residue of an L-enantiomeric oligonucleotide.

        21.   The reagent of claim 20 wherein the L-
15 enantiomeric oligonucleotide has the sequence L-
d(ACTCTC).

        22.   The reagent of claim 4 wherein L_1, L_2 or L_3 is
the residue of a heterobifunctional cross-linking
20 reagent.

        23.   The reagent of claim 22 wherein the
heterobifunctional cross-linking reagent is selected
from the group consisting of sulfosuccinimidyl 4-(N-
25 maleimidomethyl)cyclohexane-1-carboxylate,
sulfosuccinimidyl (4-iodoacetyl)aminobenzoate,
sulfosuccinimidyl 4-(p-maleimidophenyl)butyrate, 2-
iminothiolane, and N-succinimidyl S-acetylthioacetate.

30   24.   The reagent of claim 4 wherein L_1, L_2 or L_3 is
the residue of a modified nucleotide moiety containing a
reactive functional group.

        25.   The reagent of claim 24 wherein the reactive
35 functional group is selected from the group consisting
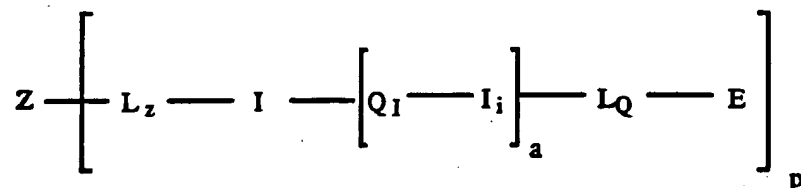of amine groups and sulfhydryl groups.

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26. The reagent of claim 4 wherein W_1, W_2 or W_3 independently contains a polycarboxylic acid group.

27. The reagent of claim 4 wherein W_1, W_2 or W_3 is independently selected from the group consisting of a residue of B4A, P4A, TMT, DCDTPA, PheMT, macroPheMT, and macroTMT.

28. The reagent of claim 4 wherein the radionuclide is selected from ^{44}Sc, ^{64}Cu, ^{67}Cu, ^{111}In, ^{212}Pb, ^{68}Ga, ^{87}Y, ^{90}Y, ^{153}Sm, ^{212}Bi, ^{99m}Tc, ^{177}Lu ^{186}Re and ^{188}Re.

29. A method of making a compound of the structure:

Structure IV

$$Z \left[L_z - I - \left[Q_I - I_i \right]_a L_Q - E \right]_p$$

wherein:

Z is the residue of an immunoreactive protein;

L_z and L_Q are independently a chemical bond or a linking group;

I is an L-enantiomeric oligonucleotide containing a contiguous sequence of from 12 to about 50 L-enantiomeric nucleotide units wherein said contiguous sequence contains one or more members of a family of homologous contiguous sequences, the individual homologs of said family containing from 12 to about 30 L-enantiomeric nucleotide units, provided that contiguous sequences of six or more L-enantiomeric nucleotide units

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of· said L-enantiomeric oligonucleotide do not hybridize
with any other contiguous sequences of six or more
contiguous L-enantiomeric nucleotide units anywhere in
structure IV;

5   Q_I is a spacing group;

a is an integer from 1 to about 6;

I_i is an L-enantiomeric oligonucleotide contiguous
sequence of from 12 to about 50 L-enantiomeric
nucleotide units, a contiguous sequence therein
10   comprising a portion of I;

E is an end· capping group; and

p is an integer from 1 to about 10;

comprising:

(i)   derivatizing Z with a precursor to a residue of L_Z
15   under conditions and for a time period sufficient to
form a covalent complex which is a precursor to a
residue of $Z-[L_Z]p$;

(ii) derivatizing· a precursor to a residue of E with a
precursor to a residue of L_Q under conditions and for a
20   time period sufficient to form a covalent complex which
is a precursor to a residue of L_Q-E;

·(iii) derivatizing a precursor to a residue of L_Q-E
with a precursor to a residue of I_i under conditions and
for a time period sufficient to form a covalent complex
25   which is a precursor to a residue of I_i-L_Q-E;

(iv) derivatizing a precursor to a residue of I_i-L_Q-E
with a precursor to a residue of Q_I under conditions and
for a time period sufficient to form a covalent complex
which is a precursor to a residue of $Q_I-I_i-L_Q-E$;

30   (v)   derivatizing a precursor to a residue of $Q_I-I_i-L_Q-E$
with a precursor to a residue of I_i and then with a
precursor to a residue of Q_I under conditions and for a
time period sufficient to form a covalent complex which
is a precursor of a residue of $[Q_I-I_i]a-L_Q-E$;

35   (vi) derivatizing a precursor to a residue of $[Q_I-I_i]a-$
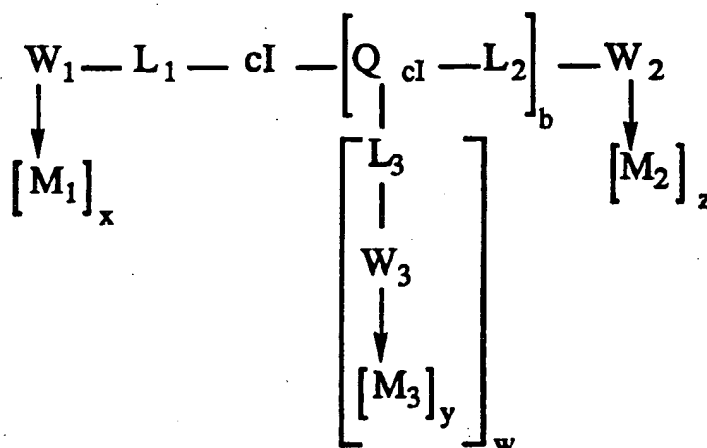L_Q-E with a precursor to a residue of I und r conditions

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and for a time period sufficient to form a covalent
complex which is a precursor to a residue of $I-[Q_I-I_i]_a-L_Q-E$;

(vii)   derivatizing a precursor to a residue of $I-[Q_I-I_i]_a-L_Q-E$ with a precursor to a residue of L_Z under
conditions and for a time period sufficient to form a
covalent complex which is a precursor to a residue of
$L_Z-I-[Q_I-I_i]_a-L_Q-E$; and

(viii)   derivatizing a precursor to a residue of $L_Z-I-[Q_I-I_i]_a-L_Q-E$ with a precursor to a residue of $Z-[L_Z]_p$
under conditions and for a time period sufficient to
form a covalent complex $Z-[L_Z-I-[Q_I-I_i]_a-L_Q-E]_p$.

30.   A method of making a compound of the
structure:

Structure V

$$W_1 - L_1 - cI - \left[Q \underset{|}{\overset{cI}{}} - L_2 \right]_b - W_2$$

$$[M_1]_x \qquad \left[\begin{array}{c} L_3 \\ | \\ W_3 \\ \downarrow \\ [M_3]_y \end{array} \right]_w \qquad [M_2]_z$$

wherein:

cI is an L-enantiomeric oligonucleotide contiguous
sequence of from 12 to about 50 L-enantiomeric
nucleotide units wherein said contiguous sequence
contains one or more members of a family of homologous
contiguous sequences, the individual homologs of which
family comprise from 12 to about 30 L-enantiomeric

nucleotide units; the L-enantiomeric nucleotide
sequences of said homologs are complementary to L-
enantiomeric nucleotide sequences of members of the set
of L-enantiomeric oligonucleotides in a co-
5   administerable targeting immunoreagent; and contiguous
sequences of six or more L-enantiomeric nucleotide units
of said complementary L-enantiomeric oligonucleotide do
not hybridize with any other contiguous sequences of six
or more contiguous L-enantiomeric nucleotide units
10   anywhere in structure V;

        Q_{cI} is a spacing group;

        L_1, L_2, and L_3 are each independently a chemical
bond or a linking group;

        W_1, W_2, and W_3 are each independently a residue of
15   a chelating group;

        M_1, M_2 and M_3 each comprise an element with an
oxidation state equal to or greater than +1, and at
least one of M_1, M_2 and M_3 is a radionuclide;

        x, y, and z are independently zero or one provided
20   that at least one of x, y, or z is one; and

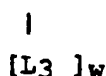   w and b are zero or an integer from 1 to about 4;
comprising:

        (i)   derivatizing a precursor to a residue of L_1 with a
precursor to a residue of cI under conditions and for a
25   time period sufficient to form a covalent complex which
is a precursor to a residue of L_1-cI;

        (ii) derivatizing a precursor to a residue of Q_{cI} with a
precursor to a residue of L_3 under conditions and for a
time period sufficient to form a covalent complex which
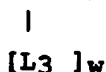30   is a precursor to a residue of Q_{cI}-$[L_3]_w$;

        (iii) derivatizing a precursor to a residue of L_1-cI
with a precursor to a residue of Q_{cI}-$[L_3]_w$ under
conditions and for a time period sufficient to form a
covalent complex which is a precursor to a residue of
35   L_1-cI-$\{Q_{cI}$-$[L_3]_w\}$;

(iv) derivatizing a precursor to a residue of L_1-cI-
$\{Q_{cI}$-$[L_3]_w\}$ with a precursor to a residue of L_2 under
conditions and for a time period sufficient to form a
covalent complex which is a precursor to a residue of
L_1-cI-$\{Q_{cI}$-$[L_3]_w$-$L_2\}$;

(v) derivatizing a precursor to a residue of L_1-cI-
$\{Q_{cI}$-$[L_3]_w$-$L_2\}$ with a precursor to a residue of Q_{cI}-
$[L_3]_w$ and then with a precursor to a residue of L_2 under
conditions and for a time period sufficient to form a
covalent complex which is a precursor to a residue of
L_1-cI-$[Q_{cI}$ - $L_2]_b$;

$$|$$
$$[L_3]_w$$

(vi) derivatizing a precursor to a residue of
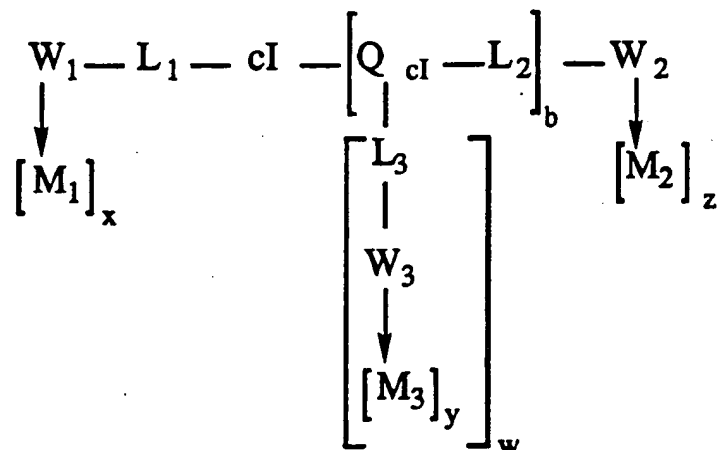L_1-cI-$[Q_{cI}$ - $L_2]_b$

$$|$$
$$[L_3]_w$$

with a precursor to a residue of W_1, W_2, and W_3 under
conditions and for a time period sufficient to form a
covalent complex which is a precursor to a residue of
W_1-L_1-cI-$[Q_{cI}$ - $L_2]_b$-W_2;

$$|$$
$$[L_3 - W_3]_w$$

(vii) derivatizing a precursor to a residue of
W_1-L_1-cI-$[Q_{cI}$ - $L_2]_b$-W_2

$$|$$
$$[L_3 - W_3]_w$$

with a precursor to a residue of $[M_1]_x$, $[M_2]_z$, and $[M_3]_y$
under conditions and for a time period sufficient to
form the compound

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$$W_1 - L_1 - cI - \left[Q \underset{|}{}_{cI} - L_2 \right]_b - W_2$$

$$\downarrow \qquad\qquad \qquad\qquad\qquad\qquad \downarrow$$

$$\left[M_1 \right]_x \qquad \begin{bmatrix} L_3 \\ | \\ W_3 \\ \downarrow \\ \left[M_3 \right]_y \end{bmatrix}_w \qquad \left[M_2 \right]_z$$

31. A pharmaceutical composition comprising a
compound of claim 3 dissolved or dispersed in a
pharmaceutically acceptable medium.

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32. A pharmaceutical composition comprising a
compound of claim 4 dissolved or dispersed in a
pharmaceutically acceptable medium.

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33. A method of treating a tumor in a mammal
comprising administering to said mammal an effective
dose of a non-radioactive targeting immunoreagent of
claim 3 in a pharmaceutically acceptable medium, waiting

15 for a time period sufficient for said non-radioactive
targeting immunoreagent to accumulate at the tumor site
in said mammal, and subsequently, administering an
effective dose of a radioactive targeting reagent of
claim 4 in a pharmaceutically acceptable medium to said

20 mammal, waiting for a time period sufficient for said
radioactive targeting reagent to accumulate at the
target site, said target site being the said non-
radioactive targeting immunoreagent accumulated at said
tumor site in said mammal.

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34. A method of diagnostic imaging in a mammal comprising administering to said mammal an imaging effective dose of a non-radioactive targeting immunoreagent of claim 3 in a pharmaceutically
5   acceptable medium, waiting for a time period sufficient for said non-radioactive targeting immunoreagent to accumulate at the imaging site in said mammal, and subsequently, administering an imaging effective dose of a radioactive targeting reagent of claim 4 in a
10   pharmaceutically acceptable medium to said mammal, waiting for a time period sufficient for said radioactive targeting reagent to accumulate at the target site, said target site being the said non-radioactive targeting immunoreagent accumulated at said
15   imaging site in said mammal and generating an image of said mammal.

35. The reagent of claim 3 wherein I is a double helix.
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36. The reagent of claim 4 wherein cI is a double helix.

INTERNATIONAL SEARCH REPORT

A.	CLASSIFICATION OF SUBJECT MATTER

IPC(5) : C07K 3/08, 15/28; A61K 39/395, 43/00

US CL :530/391.1, 402; 424/1.11, 85.91

According to International Patent Classification (IPC) or to both national classification and IPC

B.	FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/391.1, 402; 424/1.11, 85.91

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE

C.	DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,863,713 (GOODWIN ET AL) 05 September 1989, col. 3, lines 30-68; col. 7, line 40; col. 9, lines 37-43.	1-33, 35-36
Y	US, A, 5,158,880 (EVELEIGH) 27 October 1992, col. 1, lines 23-28.	1-33, 35-36
Y	WO, A, 92/08494 (TONER ET AL) 29 May 1992, see claims 9 and 11 in particular.	1-33, 35-36
Y	Journal of the American Chemical Society, Volume 113, No. 21, issued 09 October 1991, H. Urata et al, "Mirror-Image DNA", pages 8174-8175, see entire document.	1-33, 35-36

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		
		"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 MAY 1994	01 JUN 1994

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer *Jill Warden for* CHRISTINA CHAN
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such
   an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

    Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable
   claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
   of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers
   only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is
   restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
   1-33 and 35-36

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.

                            ☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

I.   Claims 1-33 and 35-36, drawn to a non-radioactive targeting immunoreagent , a radioactive targeting immunoreagent, a process of making a non-radioactive targeting compound, a process of making a radioactive targeting compound, a composition and a method of treating a tumor in a mammal, classified in class 530, subclasses 391.1 and 402 and class 424, subclasses 1.11 and 85.91.

II.   Claim 34, drawn to a method of diagnostic imaging in a mammal, classified in class 424 subclass 9.

The claimed inventions as grouped are distinct, each from the other, because they represent different inventive endeavors. The invention of Group I would not suggest the invention of Group II. Groups I and II are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.